NATURE

No. 4801 SATURDAY, NOVEMBER 4, 1961 Vol. 192

CONTENTS

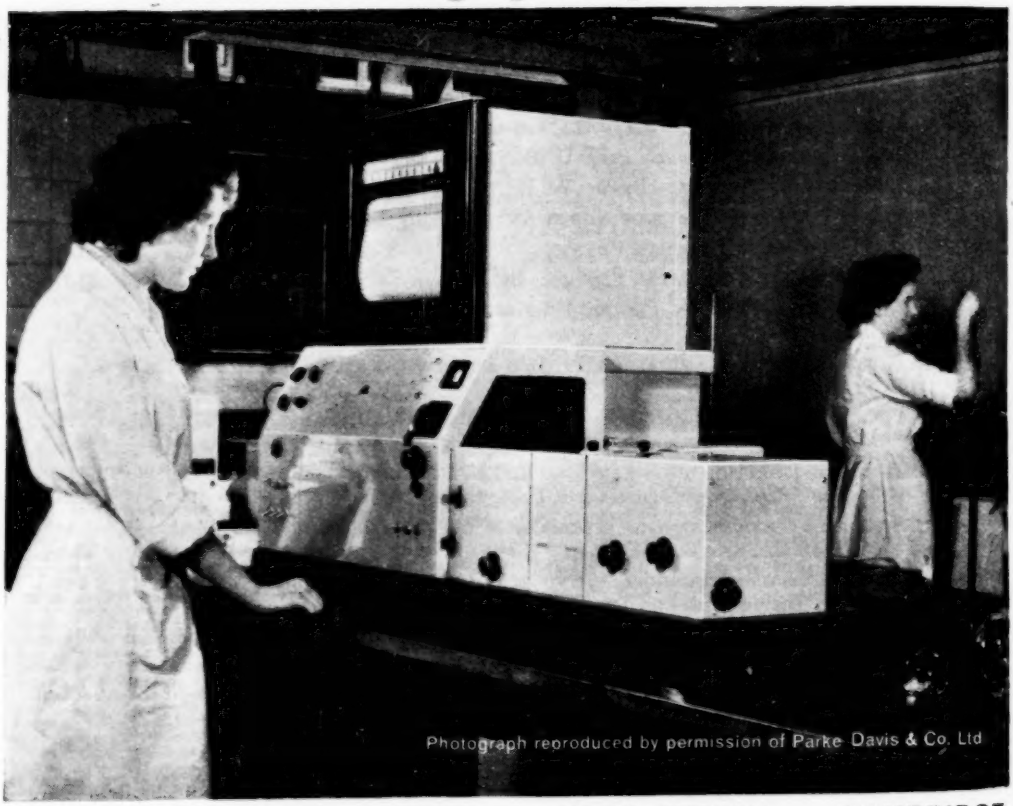
	Page
A National Youth Science Programme	387
Electrodynamics and Mechanics. By Dr. B. H. Chirgwin	389
A Study of Diabetes	391
Analytical Instrumentation. By Dr. D. T. Lewis	391
Science and Government in a Free Society. By The Right Hon. Viscount Hailsham, P.C.	393
Systematic Measurement of Fall-out in Czechoslovakia up to 1961. By Prof. W. Santholzer, G. Macku and J. Podzimek	398
Radioactive Fall-out and the Testing of Nuclear Weapons	400
News and Views	403
New Sources of Energy. By Dr. H. Heywood	407
Microwave Measurement Techniques. By Dr. G. D. Sims	408
Progress in Microchemical Methods and Techniques. By A. J. Barnard, jun., and John Y. Steel	410
Insect Polymorphism. By Prof. O. W. Richards, F.R.S.	412
Molecular Biology and Population Structure. By Prof. J. M. Thoday	414
Biology in Britain. By Prof. H. E. Street; Dr. H. V. Wyatt	416
Conquest of Hunger. By Dr. W. R. Aykroyd, C.B.E.	417
Plant Phenolics Group of North America. By Dr. V. C. Runeckles	418
Research with Tropical Plants	418
Formation of Air–Water Froths. By J. Rennie and W. Smith	419
Effect of Copper on Products of Orcinol Oxidation. By Dr. T. F. Slater	420
Participation of Imidazole in Intramolecular Hydrogen Bonding. By Prof. A. Lukton	422
Properties of Some Copper Complexes. By Dr. C. M. Harris, T. N. Lockyer and Hanneke Waterman; Dr. G. A. Barclay and C. H. L. Kennard	424
Thermal Reaction between Two Light Reactions in Photosynthesis. By Prof. C. P. Whittingham and P. M. Bishop	426
Sulphate Transport and Metabolism by *Desulphovibrio desulphuricans*. By Prof. C. Furusaka	427
Antigenicity of Penicillin and its Relation to Albumin Binding. By Dr. Toshio Torii and Dr. Yoshihi Kohoriuchi	429
Inherited Casein Variants in Cow's Milk. By Dr. R. Aschaffenburg	431
Comparison of Transduction versus Transformation of Linked Markers in *Bacillus subtilis*. By Erela Ephrati-Elizur and Maurice S. Fox	433
Extracellular Esterase of Group *A* Streptococci. By A. H. Stock, José Uriel and Pierre Grabar; A. H. Stock and José Uriel	434
A Cell for rapidly measuring Restriction to Diffusion of Macromolecules through Agar Gels of Various Pore Sizes. By G. K. Ackers and R. L. Steere	436
Autoradiography using Melted Nuclear Emulsion. By Dr. A. C. Kulangara	437

(*Continued overleaf*)

UNICAM

This is the SP.700 Spectrophotometer. One of the most advanced analytical instruments of its kind. Precise—versatile—reliable—made for analysis through a spectral range of Ultraviolet, Visible and Near Infrared. The SP.700 records a detail of the composition of unknown mixtures—detects minute traces of the most elusive elements—completes in minutes a task which would take hours or weeks by other methods. Used by scientists in the great University and Industrial Laboratories of the world. Made by Unicam Instruments Ltd., York Street, Cambridge.

SP.700 Recording Spectrophotometer



UNICAM INSTRUMENTS LIMITED YORK STREET · CAMBRIDGE

LETTERS TO THE EDITORS

Page

COSMOLOGY

Dirac's Cosmology and Mach's Principle.—Prof. R. H. Dicke; Prof. P. A. M. Dirac, F.R.S. . . . 440

SPACE SCIENCE

Direct Measurement of Interplanetary Dust Particles in the Vicinity of Earth.—C. W. McCracken, W. M. Alexander and M. Dubin 441

ASTRONOMY

Exact Determination of the Lunar Mass by means of Selenoid Satellites.—Dr. E. T. Benedikt . . 442

PHYSICS

Radioactivity in Rainfall during September 1961 following the Russian Nuclear-Weapons Tests of September 1961.—Keith Boddy 443

Nuclear Spin of Protoactinium-231 from Hyperfine Splitting.—E. W. T. Richards, A. Ridgeley, N. J. Atherton and H. S. Wise 444

Rotational Analysis of $^{63}Cu^{35}Cl$ Bands.—Prof. R. K. Asundi, P. Ramakoteswara Rao and J. K. Brody . 444

Electron Paramagnetic Resonance and Antiferromagnetism in $LaCrO_3$.—I. Weinberg and P. Larssen 445

GEOLOGY

Potassium–Argon Ages at Broken Hill, Australia.—Dr. J. F. Evernden and Dr. J. R. Richards . . 446

METALLURGY

Difference between High- and Low-Stress Fatigue.—N. E. Frost 446

CRYSTALLOGRAPHY

Lattice Rotation at Slip Band Intersections.—Dr. A. S. Argon and Prof. E. Orowan, F.R.S. . . . 447

CHEMISTRY

Requirements of an Ice Nucleus.—G. R. Edwards and L. F. Evans 448

Evaluation of Boundary Layer Thickness in Electrolytic Systems from Decay of Anode Concentration Polarization.—Leonard Nanis 449

Pyrolytic Carbon Formation from Carbon Suboxide.—B. C. Banerjee, T. J. Hirt and Prof. P. L. Walker, jun. 450

BIOCHEMISTRY

Changes in Adenine Nucleotides in the Muscles of Some Marine Invertebrates.—Ken-ichi Arai and Tsuneyuki Saito 451

Effect of Charcoal on the Behaviour of Adenine Nucleotides in Ion-Exchange Chromatography.—U. Pethiyagoda 452

Changes in the Concentration of Ribonucleic Acid during Wound Tissue Regeneration.—Martin B. Williamson and Wilhelm Guschlbauer 454

Phosphorylserine as a Constituent of Phosphoproteins from Plasma of the Laying Hen.—P. J. Heald . 455

Distribution of Ribonucleic Acid in Subcellular Fractions of Various Regions of the Central Nervous System of the Rat.—T. Borkowski, S. Harth, R. Mardell and Prof. P. Mandel 456

Cystine Content in Hair of Children as influenced by Vitamin A and Animal Protein in the Diet.—Tatsuo Koyanagi and Teru Takanohashi 457

PHYSIOLOGY

Amounts of Oxides of Nitrogen and Carbon Monoxide in Cigarette Smoke, with and without Inhalation.—C. Bokhoven and H. J. Niessen 458

Sodium and Potassium Content in the Muscles of Hibernating Animals.—Dr. Einar Eliassen and Helge Leivestad 459

Diuresis due to Stress in Cattle.—R. S. Anderson . 460

Effects of Thymectomy on the Resistance of Rats to Drowning and Histamine Stress.—Dr. A. S. Weltman and A. M. Sackler 460

Plasma Iron and Iron-binding Capacity in the Pregnant Rat and Rabbit.—E. H. Morgan 461

Page

Importance of Cell Division for Antibody Production in an *in vitro* System.—R. W. Dutton 462

PHARMACOLOGY

Effect of Derivatives of L-Cysteine on Growth of *Leuconostoc mesenteroides* (8042).—A. B. K. Njaa . 463

Second-Generation Toxicity of Malathion in Rats.—Prof. W. Kalow and A. Marton 464

5-Hydroxy-3-indoleacetamidine: a New Type of Blocking Agent against 5-Hydroxytryptamine.—Dr. Laszlo Gyermek 465

HISTOCHEMISTRY

Variation in Lyoglycogen in Starved and Normal Guinea Pigs.—W. J. C. Wilkinson and J. H. Kugler 466

HÆMATOLOGY

Specific Antigens of Human Leucocytes and Thrombocytes.—Nechama Gilboa-Garber and Dr. D. Nelken . 466

PATHOLOGY

Cultivation of Monolayer Cultures of Frog Renal Cells.—Dr. Walter Auclair 467

Acylase Activity of Ascites Tumour Cells on Dichloroacetyl Derivatives of Amino-acids.—Yukio Kameda, Katsuhiko Matsui, Yukio Kimura and Etsuko Toyoura 468

BIOLOGY

Chloride Regulation in *Triops*.—Gwyneth Parry . 468

Effect of Feeding Griseofulvin on Rate of Growth of Chicks.—Dr. W. P. Jaffe 469

Feeding of Bamboo Flowers to Laboratory Rats and Mice.—V. Mahadevan, Dr. S. S. Negi and O. N. Agarwala 470

Classification of Fossil Microplankton.—Dr. C. Downie, G. L. Williams and Dr. W. A. S. Sarjeant . . 471

Alteration of Permeability of *Lactobacillus plantarum* caused by Chlorpromazine.—Dr. Helene A. Nathan 471

A Chamber for culturing and collecting the Nematode *Panagrellus redivivus*.—E. J. Winkler and Prof. David Pramer 472

Periodic Acid–Schiff Technique for the Demonstration of Extra- and Intra-cellular Stages of Gregarine and Coccidian Protozoa.—Dr. J. B. Jennings . . 473

ENTOMOLOGY

Evaluation of the Activity of Some Diphenyl Compounds on Winter Eggs of the Fruit Tree Red Spider.—J. Meltzer 474

A Technique for exposing Individual Flies on Insecticidal Deposits.—H. C. M. Parr and J. E. H. Grose . 475

GENETICS

Primary Embryonal Sex Ratio in Normal Pregnancies determined by the Nuclear Chromatin.—Prof. F. E. Szontágh, A. Jakobovits and Ch. Méhes . . 476

A Rare Phenotype within the *Gm* System.—K. Henningsen and Jørgen C. Nielsen 476

Glucose-6-Phosphate Dehydrogenase Activity in Papuans.—Dr. B. P. K. Ryan and I. C. Parsons . 477

VIROLOGY

Cyclic Events in the Viral Cycle.—Dr. André Lwoff, For.Mem.R.S., and Marguerite Lwoff 477

Multiplication of Mouse-adapted Influenza Virus in Mouse Lungs after Infection with Very Low Doses.—F. Link and J. Raus 478

AGRICULTURE

Nitrogen-fixing Blue-Green Algæ in Australian Rice Soils.—Dr. J. S. Bunt 479

Control of the Army Worm on Grazing in Kenya.—J. A. Bullock and D. C. Bond 480

SOIL SCIENCE

Influence of Some Organic Salts on the Absorption of Potassium by Vermiculite.—Prof. M. M. Mortland . 481

DIFCO

microbiological reagents and media

THE ONLY COMPLETE LINE

DETECTION OF THE SALMONELLA-SHIGELLA GROUP

Bacto-SS Agar

is a selective medium especially designed for use in isolation of fastidious *Shigella* and *Salmonella* strains. The selective action of this medium restrains to a large extent the development of coliform bacteria with minimum restriction of fastidious strains of the pathogens. Because of the inhibitive action of the medium of coliform bacteria, it is possible to inoculate the medium heavily with faeces thereby greatly increasing the chance of positive isolations from samples containing very few pathogens.

Bacto-Bismuth Sulphite Agar

is a highly selective medium for isolation of *Salmonella typhosa*. The unusual selective properties of this medium permit the use of large inocula of faeces and other suspected material without overgrowth of extraneous intestinal bacteria.

Bacto-MacConkey Agar

is an excellent differential medium for use in conjunction with Bacto-SS Agar and Bacto-Bismuth Sulphite Agar. This medium supports rapid and luxuriant growth of even the most fastidious strains of the typhoid-dysentery group.
Although MacConkey Agar does not inhibit coliform bacteria it does afford excellent differentiation of colonies of pathogens from those of the lactose fermenting bacilli.

Bacto-Tetrathionate Broth Base
Bacto-Selenite Broth

enrichment media for isolation of intestinal pathogens. These are excellent aids in the detection of carriers and examination of other materials for members of the *Salmonella-Shigella* group.

Please send for the latest technical information.

BTL complete laboratory service

BAIRD & TATLOCK (LONDON) LIMITED, CHADWELL HEATH, ESSEX, ENGLAND.
Branches in London, Manchester and Glasgow.

TAS/DO.12

Ultra-Violet & Infra-Red Sampling Instruments

ULTRA-VIOLET ABSORPTION CELLS
VARIABLE path-length cells for liquids
FIXED path-length cells for liquids

KBR EVACUABLE DIE
for making 13, 15 and 16 mm. Discs

HYDRAULIC 30-TON PRESS
especially designed for KBr Disc Technique

Rotating Electrode (Solution Excitation) Spark Unit

INFRA-RED ABSORPTION CELLS
VARIABLE path-length cells for liquids
FIXED path-length demountable and sealed cells for liquids
MICROCELLS for liquids
GAS cells in pyrex and metal

HIGH-SPEED VIBRATION MILL
indispensable for KBr Disc Technique
available for 230 volt 50-cycle and 110-volt 60-cycle mains

ROCK-SALT POLISHING KIT

RIIC Sampling Instruments are marketed by the leading spectrometer manufacturers of the world. Descriptive literature on application.

RESEARCH & INDUSTRIAL INSTRUMENTS CO.
116 Lordship Lane, London, S.E.22 Telephone: Forest Hill 2281/2

A NATIONAL YOUTH SCIENCE PROGRAMME

RECOGNIZING the great strides being taken by science to-day; the fact that, according to Sir Bernard Lovell, of all the scientists who ever lived 90 per cent are with us to-day; and, above all, universal acknowledgment of the fact that all and sundry are now in some way or another responding to the impact of science on themselves and on society (whether conscious of it or not), one must admit that there is need for everybody to learn something about what is behind this great and rather sudden social upheaval.

Science yields power: it renders physical power to industry and travel, further means of interpretation in the various arts, more facilities for enjoying one's leisure; but it is to be hoped that it will never succeed in giving power to the few who might use it for controlling the many. Yet that risk seems very real to-day, due mainly to the widespread lack of understanding of the true aims and objectives of science. As Mr. Dean Rusk recently said: "Power is not a matter of arms alone. Strength comes from education, fertile acres, humming workshops and the satisfaction and pride of peoples". Strength assuredly comes with education, and in this science must play a much greater part from the earliest years of a child's life. This has also been emphasized recently by Lord Hailsham, the Minister for Science (see p. 393 of this issue).

For many years one referred to pure science and applied science, this eventually being altered to fundamental and applied science. Gradually it has become recognized that there is no hard and fast line of demarcation between the two—that one inspires the other, and that fundamental science is not entirely basic to applied science and vice versa. To-day, one hears of science and technology; perhaps the day is not far off when the implied distinction between these two will disappear. Accepting, perhaps with some reservation, Murray Schoonraad's statement that "to-day's science is to-morrow's technology" (*Nature*, **191**, 848; 1961), it would seem that there is no real distinction between science and technology, but that over a period of time one merges into the other and that the length of this period is quickly becoming shorter. Science should eventually, therefore, be recognized for what it really is, namely, the search for the truth (using its own peculiar means), the exposition of the truth and its application wherever possible to man's needs and comforts.

The 'balance of power' periodically changes even within the ranks of the scientists themselves, such changes being inspired by popular appeal or curiosity or sudden recognition of profitable application. For example, in the Victorian era, Darwin and his supporters stirred the world with the conception of the origin of species. As these ideas appeared to strike at the very roots of the religious faiths and beliefs of millions of people at that time, one can easily imagine how science (in this case, biology) was brought to the foreground in public debate, discussion and sometimes heated argument as exemplified by the Huxley–Wilberforce contretemps at Oxford and, much later, the growth of so-called Fundamentalism in the United States which, starting as a denominational interest, eventually became a national concern when, in 1925, a teacher in Dayton, Tennessee, was tried and convicted for teaching the principles of evolution in a State school. Thus, for a time, science made a spectacular appearance before a world-wide audience.

One can think of other scientific spectacles, those of to-day obviously being feats in space science. But such spectacles can sometimes do more harm than good to science itself; for it would be undesirable to have a scientific *élite* in any community, such as is now developing in the U.S.S.R. Science is actually proceeding on a very broad front, and many of its facets, highly important though they are, can never be spectacular. So it can be only a very small majority of people who recognize this general advancement; and, to-day, no one person can be expected to understand all of it. Even now it would be a very small percentage of those who have not read biology to a fairly advanced level in school who could give the names of the scientists who discovered penicillin or insulin. So clumsy is the way in which science is presented to the public that it is unlikely that Fleming's name will ever become such a household word as that of Gagarin. Macromolecular biology is now making great strides in the field of scientific endeavour; but it will be some time before the non-scientist will be able to understand and appreciate this.

This does not imply that all thinking people are not aware of the need for a much wider and deeper appreciation of science and its impact on human society. The main problem is how to set about it. It seems better to acknowledge the fact that among adults only those who wish to know something about this 'Age of Science' will take the trouble to learn. This minority is fairly well catered for already. In Britain, the British Association for the Advancement of Science, the Workers' Educational Association, the extra-mural departments of the universities, many local authorities, the British Broadcasting Corporation and television authorities are doing excellent work to enlighten those who wish to learn.

This is by no means enough, for it is not only the mature grounds which need attention but also the newer, uncultivated yet potential fertile soil—men and women of to-morrow, who are recognizable as soon as they are able to speak.

Governments, local authorities, industry, etc., are bringing pressure to bear in an all-out attempt to increase, widen and improve the standards of science teaching. In some cases, no doubt, the results are proving disappointing and mediocre, this being due

to many conditioning factors, the chief of which is the dearth of science teachers, unsuitability of some of these, lack of space and equipment, finance, and so forth; but above all the time-factor in school terms and hours.

Science teaching seems to have become too rigid, especially in its attitude towards the applications of science. This may be due to a schism in thinking of science as a cultural discipline in contradistinction to science as a practical need. (The examination systems may be largely responsible for this.) Heuristic methods of teaching, as practised and advocated by H. E. Armstrong earlier in this century, served a useful purpose at the time in that they pointed the way to an effective means of teaching the scientific method of experimentation. But such methods aim largely at training potential scientists, and though they inspire some children who have no intention of becoming professional scientists they might well repel others. They are, therefore, not entirely satisfactory if we are to capture the imagination of the majority of the very young in the appreciation of science. The practical application of *A* to *B* may not be so interesting as the application of *A* to self, for what touches the heart of the layman is the effect scientific progress is having on himself—that is, the practical applications of science to man, his health, his job, his creature comforts and, above all, his future. The laboratory is not the only room where science can be taught: it is not necessary to dissect a rabbit in order to learn about the bones of the mammalian skeleton, neither is it necessary to use earthworms with the ancillary apparatus to understand about respiration in animals. Many observations and experiments can be carried out on the pupil's own body, thus becoming more interesting. None of this need undermine the ultimate aim of treating science for what it is, namely, a cultural, and therefore not solely a technical, discipline.

Where, therefore, are the other and earliest grounds for successful scientific seeding? When does a child reach the critical point when he might become inspired with things scientific? The first lies within the ægis of the primary school (many of which have no laboratory), and the second depends on how successful is the seeding in the first. It was with the view of considering how the initial seeding could best be accomplished that the British Association for the Advancement of Science convened a conference, during September 29–30, jointly with the Association of Teachers in Colleges and Departments of Education, on "The Approaches of Science in the Primary School".

At this conference, Mr. N. Isaacs said that the "approaches" in this case are through a disciplined way of thinking, a way encouraged by a definite method of exploration of the physical, biological and psychological worlds. The natural learning processes of the child are akin to the development of scientific thinking—exploration, collecting and piecing together experiences, building up these into a connected scheme of knowledge and foresight. Such processes can be observed in the developing child, and become most marked when the child, in its third or fourth year, has sufficiently mastered language to discover the way of question-forms. The method of seeding should be one of accentuating and directing the natural learning process. This would, it is hoped, be achieved by creating in the primary schools specific environments aimed at developing the individual's interest by setting the scene, catching his innate curiosity and spurring him on to observe and bring forward the right kind of question and hence an attitude to exploration. So, by the time he enters the secondary school, the child is competent to know his world, capable of planning and ready for things scientific. Given an interest in science he will need nothing but opportunity to develop it. However, unless the child is deeply and naturally instilled with the human implications of his knowledge in the primary school, his approach to science teaching in the secondary school will not be satisfactory.

Much more can be done also outside school hours. A great deal has already been done in the United States in this highly important field of extra-mural endeavour. By 1940 some seven hundred 'science clubs' had been founded there. The movement was somewhat retarded by the Second World War; but the project gained momentum during the post-war years, and now there are about 25,000 science clubs of which approximately 500 are outside the United States. Through what is termed 'Talent Searches' individual boys and girls showing special aptitude are encouraged, and it is now claimed that 95 per cent of those winning honours in the Science Talent Search proceed to the universities. According to Science Service's *Growth of the Science Youth Program* (October 10, 1959), honours were awarded to about 10 per cent of the entries and about 50 per cent of these eventually gained doctorates. Yet it is to be hoped that those who were not so selected are now progressing through life with a better understanding of science than they would otherwise have had. If so, then Science Service (Washington) is doing excellent work. This Service also inaugurated 'Science Fairs', first on a local and regional basis. By 1950 these had become so numerous that the National Science Fair was instituted. In 1958 this Fair became international through the entry of finalists from Japan's National Fair.

This project could well be followed elsewhere, as indeed it is in Britain, though two other methods of somewhat similar pattern might be considered. First, a central organization might be set up to encourage and direct the attention of science teachers to possible extra-mural activities aimed at young children and teenagers in most branches of science—astronomy, the physical sciences, archæology, natural history, engineering, agriculture, horticulture and so forth. Such an organization might invoke the aid and advice of national and local scientific societies. Thus it should be possible to encourage school teachers to become more flexible in their teaching by organizing extra lectures and exhibitions, and above all, by making available opportunities for practical work during term-time at specific centres.

These activities might, though not necessarily, be divided into age-groups, but should on no account be confined to those pupils who are aspiring to the science sixth form.

It can be taken for granted that, once established, such a scientific centre would be able to rely on much practical assistance from local industry, though that would depend considerably on the personality and tact of those running the centre. In any event, the time seems ripe for academic and industrial research centres to open up their laboratories, workshops and even factories more often than they do for organized visits by school children. Museums play their part; but this is not enough. Of course, a great deal is already being done, but much more is needed, for this will help to inspire interest among the many who may never become professional participants in future years; and it must be recognized that these are the laymen of the future so far as science is concerned.

Certain sections of industry actually do arrange campaigns, and send their representatives to schools and universities; but their object is clearly to catch the future scientists, and in any event they, and others, might do still better by 'selling' themselves on their own premises rather than in the school lecture theatre.

A second method of approach takes into account the fact that it is asking a great deal of any school teacher to take on still further responsibility, especially out of school hours, terms or grounds. Yet much could be done, as in the Boy Scouts and Girl Guides movements; that is, not only outside school hours but also away from the daily school teacher: with all due respect to him, a change of senior personnel might prove stimulating.

The British Association is doing much towards educating youth. During the recent annual meeting at Norwich there were three young people's lectures, a schools exhibition, the President's tea party for student exhibitioners and the annual session devoted to "Science in the Sixth Form" organized by Section X. This is good and points the way to further endeavour with still younger children in view. Nevertheless, it must be recognized that such events are confined to a few days each year, to the comparatively small number of large towns and their catchment areas and to a fairly advanced age-group. With its lecture services, young people's programmes, international youth science conferences, area committees, science fairs, etc., the British Association has made increasing and comprehensive contributions to the development of more widespread scientific education. But it would be a greater step forward if such a body as the British Association made a survey of the needs and potentialities of the whole country, for there is much to be found—the entire country is not lying fallow. There are numerous local scientific societies, many of them, it is true, confining themselves to natural history and archæology; but there they are, and some have junior sections. Then what always seems to be overlooked is the fact that there are many individual enthusiasts and sometimes small groups of enthusiasts who are interested in a very practical way in most branches of science. It is often stated that the days of the 'amateur' scientist are gone; but we believe this is far from the truth. It was Will Hay who made very important contributions to astronomy using his 6-in. Cooke refractor mounted in his home-made observatory; and by profession he was a stage and screen actor. We believe there are many of his type, in various scientific disciplines, cultivating their interests to-day.

Most of these local societies, clubs, and individual enthusiasts appear to depend mainly on themselves. Yet it is they who might be prepared to form the nuclei for still further local endeavour with the young as their main objective. They might well take into consideration the lines of approach suggested by Sir Eric Ashby in his British Association Granada Lecture in 1960. He proposed that an understanding of science could be imparted by emphasizing the importance of technique in science, for the ordinary man is a craftsman and he appreciates craftsmanship. This might well be extended to the children and youth of to-day.

Such local individual and collective movements might be loosely unified under a sponsoring body such as the British Association, which is not only well equipped with the necessary contacts but also has the nucleus of the necessary staff. Such a sponsoring body, set up on a national scale but using and inspiring local men and women, could offer to the boys and girls of Great Britain the encouragement in scientific activity and appreciation that the National Playing Fields Association, with the invaluable patronage of the Duke of Edinburgh, is giving them in sport and recreation.

ELECTRODYNAMICS AND MECHANICS

Electrodynamics of Continuous Media

By L. D. Landau and E. M. Lifshitz. Translated from the Russian by J. B. Sykes and J. S. Bell. (Course of Theoretical Physics, Vol. 8.) Pp. x+417. (London and New York: Pergamon Press, 1960.) 84*s*. net.

Mechanics

By L. D. Landau and E. M. Lifshitz. Translated from the Russian by J. B. Sykes and J. S. Bell. (Course of Theoretical Physics, Vol. 1.) Pp. vii+165. (London and New York: Pergamon Press, 1960.) 40*s*. net.

THE titles of the volumes in this series cover a vast range of topics and there seems to be little in physics on which the authors are not very well informed. The volume on electrodynamics conveys a sense of mastery of the subject-matter on the part of the authors which is truly astonishing. I myself have read only one other volume of the series and cannot lay claim to any specialized knowledge of any of the topics dealt with. These remarks, therefore, cannot criticize any details of the physics discussed in this volume.

The broad lines adopted by the authors become clear very quickly. Maxwell's equations are assumed

XUM

to hold on a microscopical scale and all macroscopical results are derived by a process of taking averages. Forces and energy relations are obtained by a systematic use of the concepts of thermodynamics—there is no place here for the transport of current filaments from infinity and similar 'elementary' devices. The ambiguities of sign sometimes associated with energy expressions are firmly resolved and all such quantities put into their proper perspective as thermodynamical quantities. But such matters are not the chief concern of this volume.

With the help of straightforward ideas of symmetry, linear dependence (and contour integration) the basic ideas of electromagnetism are developed to apply to a long list of topics. These include electrostriction, piezoelectricity, ferroelectrics, the Hall effect, contact potential, electrocapillarity, thermoelectricity, gyromagnetism, ferromagnetism, superconductivity, eddy currents, the skin effect, magnetohydrodynamics, dispersion, wave-guides, double refraction, etc. This list is by no means complete. The standard of explanation is admirable, being sufficient to give a moderately informed reader a grasp of the principles concerned, both physical and theoretical, without elaborate detail. The approximations which must be made to make a theoretical treatment feasible are explained and the theory developed starting from the field equations in most cases. It is the physical insight which accompanies and guides this development that conveys the sense of mastery mentioned here.

To-day the division of the subject-matter of physics into heat, light, sound and properties of matter is being superseded; this book will accelerate this change. In fact, one of the impressive features of the book is the way in which diverse phenomena, listed above, are shown to be related through the dependence of ε (permittivity), μ (permeability), or σ (conductivity) on the frequency of the radiation. Phenomena, which in elementary physics are explained by a closed and mathematically elegant theory, here appear related as a result of expanding ε, say, in terms of the frequency. The general effect is to demonstrate the full power and generality of electrodynamic theory in a way seldom achieved in a textbook.

The translators are to be congratulated on the effectiveness of their translation; the language is direct and could well be original English text. There are surprisingly few typographical errors, about a dozen were noticed, most of these being the omission of a letter or a symbol. Throughout the volume there are references from time to time to modern work, mostly in Soviet journals, a large proportion being available in translation. In short, *Electrodynamics of Continuous Media* is a book which can be thoroughly recommended to every physicist.

In the history of science it has always required a great intellectual effort to break free from established modes of thought and to establish a new outlook, exemplified in the change from the Ptolemaic to the Copernican view of the universe. To-day we are in the midst of a similar change with the advent of modern physics, and the volume on mechanics will contribute to the establishment of a new outlook.

Although the subject-matter is, of course, Newtonian mechanics, the basic outlook is different from the traditional Newtonian outlook. From the outset Hamilton's Least Action Principle is taken as the fundamental law governing all motion; an inertial frame is one for which space and time are isotropic and homogeneous, and the basic transformation is the Galilean transformation between two inertial frames. Considerations of symmetry lead to the law of inertia, and the equivalence of two inertial frames leads to the introduction of a coefficient identified as the mass of a particle. A system of particles which interact with one another but with no other body requires an addition, $-U$, to the sum of the Lagrangian functions of the particles to give the Lagrangian of the whole system. This function U, depending on the co-ordinates only (non-dissipative systems only ?) is the potential energy. There is no indication of how to find the form of U in any given case. The use of the equations of motion now introduces the concept of a force and we arrive at Newton's Second Law of Motion. Throughout this part one feels that without the familiarity gained from the traditional outlook and the associated intuitive appreciation of mass, force, etc., it would be difficult to appreciate the proper significance of many of the results. In some of the succeeding chapters these intuitive ideas are still necessary to 'feel what is going on'—the escape from traditional views is not complete.

On one point of the dynamics of a rigid body the treatment is not quite satisfactory. On p. 109 it is said that the equation for the rate of change of angular momentum "May be regarded as Lagrange's equation . . . for the 'rotational co-ordinates'.". Apart from the inverted commas it is not pointed out that there are no such co-ordinates, that a component of angular velocity is not, in general, the rate of change of any one angle, as is shown afterwards in equation (35.1). This topic should, strictly, be treated as a non-holonomic constraint according to the methods of a subsequent section. Nevertheless, this is a familiar point and the reader already knows the results. It does not affect anything else in the book.

When the reader has adjusted himself to the new outlook then the rest of the book, the greater part, is a vindication of the effort of making this adjustment. The scope and power of the methods used are made abundantly evident in fields of importance to physicists; little time is spent on artificial classical problems. The theory of collisions between particles is developed in a form in which physicists need it; this is followed by chapters on small oscillations, the motion of a rigid body, and finally Hamilton's equations, Poisson brackets, Liouville's theorem, Hamilton–Jacobi equation, etc. This last chapter lays the foundations for the transition to quantum mechanics.

In undergraduate courses on dynamics the treatment of small oscillations often leaves the impression that the theory is self-contained without room for development. Here it is clear that the familiar theory is one stage in the approximations necessary for the discussion of such motions. The development to anharmonic and non-linear oscillations follows naturally. This is typical of the way in which the reader is shown that classical mechanics is still capable of development. The illustrative problems are very carefully chosen and the accompanying solutions are admirable for conciseness, the salient points only being given.

It is a remarkable achievement to write a text on advanced analytical dynamics in the compass of 165 pages and to produce such a stimulating account. The translation is excellent and errors of typography are very few and far between.

B. H. Chirgwin

A STUDY OF DIABETES

Diabetes in an English Community

A Study of Its Increase and Natural History. By Joan B. Walker and David Kerridge. Pp. 46. (Leicester: The University Press, 1961.) 7*s.* 6*d.*

A VALUABLE survey of the incidence and natural history of diabetes in a rural parish (Ibstock in Leicestershire) has been made by Dr. Joan Walker of Leicester Royal Infirmary and David Kerridge of the University of Sheffield.

The inquiry was initiated in 1955 by the British Diabetic Association in an attempt to arrive at a numerical evaluation of the problem of diabetes in Britain. The figures available from the General Register Office vary from two to four diabetics per thousand of the population, but these figures refer to known diabetics. It was well known from the United States and Canada that, in previous surveys, as many unknown diabetics were found as those already diagnosed.

Many of the methods used by other investigators depended on the study of clinics, schools, factories, prisons and other such institutions, which are special groups with age and sex selection, while detection drives, although not restricted to special age or occupation groups, depend on voluntary initiative and tend to have low response-rates, and therefore allow too much room for bias. Throughout the year 1956, these ways of determining the incidence of diabetes in Britain were discussed. It was essential to find a method which would be practical, easily applied elsewhere, and be, at the same time, accurate. It was agreed that no census of known diabetics attending clinics could produce the information required. As it was most important for the purposes of the investigation that follow up should be possible, it was finally decided to investigate a compact stable community.

Because the diabetic clinic at Leicester Royal Infirmary served the City and whole County of Leicestershire, and because some of the Leicestershire villages had exceptionally static populations, it was decided to investigate a village community with the view of its becoming part of any larger national or international survey which might be undertaken later.

The objectives of the inquiry were as follows: to establish the number of diabetics in a natural area where the population is static, to discover the undiagnosed cases of diabetes, to investigate the possible hereditary factors; to investigate the possible ætiological factors of physique, diet habits, stress, occupation, and parity in women; to follow up the cases of glycosuria discovered by the investigation, if possible, for as long as twenty years, and to watch for new cases developing in the area; to repeat the whole survey at a future date and to repeat it also in another area of England for comparison or contrast.

Every person living in the village who was over the age of five years was asked to supply a specimen of urine and to answer a questionnaire. Altogether 2,071 males and 2,034 females were tested making an 81 per cent response of the population over the age of five years. Details of thirty-three previously diagnosed cases were collected from general practitioners' and clinic records. So far as could be determined the 19 per cent of non-co-operators were not different in age or other environmental factors from the rest, and in calculating rates, it was assumed that they were a random sample of the whole population.

The results show that there are about thirteen diabetics per thousand of the population, half of whom are asymptomatic. The relationship of diabetes to various social factors was examined. Plans for a follow-up study of the population are also described in the booklet.

ANALYTICAL INSTRUMENTATION

Physical Methods in Chemical Analysis

Vol. 1. Edited by Walter G. Berl. Second Edition. Pp. xiv+686. (New York: Academic Press Inc.; London: Academic Press Inc. (London), Ltd., 1960.) 19 dollars.

ADVANCES and refinements in analytical instrumentation must, of necessity, call for periodic reviews of those publications which form such an essential part of that ever-expanding physicochemical noosphere from which the modern scientist draws both inspiration and knowledge. This present revision (Volume 1) of a well-known authoritative treatise on instrumental methods in chemical analysis belongs to this category.

The more elementary student may find the advanced and detailed form of presentation somewhat bewildering, but as his knowledge increases and he joins the ranks of the experts, he will undoubtedly appreciate the lucidity with which the various specialist contributors have described those practical and theoretical problems which are concomitant with the complex field of contemporaneous instrumentation. This is, however, a book which has been written by experts for experts, and, at the academic stage, it will undoubtedly be of the greatest assistance to honours graduates and research workers. Most modern instrumental methods are described with much attention to practical laboratory detail. Nearly a quarter of the 670-page work is devoted to X-ray diffraction techniques as applied to powders, metals and polymeric fibres. Spectrophotometry, emission spectrography and infra-red spectroscopy are given comprehensive treatment in chapters of about 80 pages each. The diagrammatic illustrations are clear and well annotated and each chapter concludes with an extensive bibliographical reference section.

Later sections deal with Raman spectra, measurements of refractive index, mass spectrometry, and electron microscopy and diffraction. The latter technique is particularly valuable for those crystallographic investigations on surfaces where the affected layer is too thin to be detected by X-rays. The section on electron microscopy gives some extremely interesting reproductions of electron micrographs of films of various materials.

The applications and design of simple and tandem mass spectrometers are described in some detail. Reference is made to the usages of such instruments in the analysis of chemical mixtures, the leak-testing of evacuated vessels, measurements of appearance potentials and related thermal quantities, reaction kinetic studies, etc.

This new edition undoubtedly provides up-to-date information on progress in the development and application of physical instrumentation and will be eagerly acquired by all chemists having an interest in the theory and practice of these specialized analytical techniques.

D. T. LEWIS

Materials for Nuclear Engineers

Edited by Dr. A. B. McIntosh and T. J. Heal. Pp. ix+373. (London: Temple Press, Ltd., 1960.) 75s. net.

WRITTEN by specialists on the United Kingdom Atomic Energy Authority staff, this handbook on nuclear materials should prove invaluable as a reference volume not only to nuclear engineers but also to many scientists and technicians working in the nuclear power field. The materials covered are uranium, plutonium and thorium metal fuels, together with their more useful ceramic compounds: magnesium, beryllium and zirconium canning materials and graphite as the most important solid moderator. The occurrence, extraction, fabrication, physical and mechanical properties, compatibility in various reactor environments and behaviour under irradiation are discussed for each material. The data are presented in suitable tabular and diagrammatic form and have been selected as the best available from published information to mid-1959.

It is regrettable that space could not be found to include stainless steel canning materials, ferritic steels which are of current interest for pressure vessels, and beryllia, which is the most promising alternative solid moderator to graphite, particularly for high-temperature gas-cooled reactors. Equilibrium diagrams are given for sixteen plutonium alloy systems and it would be valuable to have diagrams also included for the technologically important alloys of uranium and thorium.

As this is a rapidly expanding field, new or revised data, especially on the irradiation behaviour of fuels, are continually forthcoming, and it is to be hoped that suitably revised editions will be produced in good time. In particular, the nuclear engineer will need to be kept up to date on the progress of ceramic and cermet fuels which are becoming of increased importance.

Neuro-Psychopharmacology

Proceedings of the First International Congress of Neuro-Psychopharmacology, Rome, September 1958. Edited by P. B. Bradley, P. Deniker and C. Radouco-Thomas. Pp. xv+727. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1959.) 135s.

THOUGH neuropsychopharmacology might be shortened as a word, the book deserves no shortening, for the contributions are uniformly of a high standard, and the book reflects credit on all associated with it. Its encyclopædic range and its great quantity of recorded facts make it an essential reference book to every worker in the subject. The editorial work has been so well done that the great quantity of material is everywhere clear and orderly. Two indexes are provided, and it is well printed and bound. The discussions given are apparently second thoughts, for they are terse and meaningful, much better than the obscure muddle of the truly verbatim record.

Comment on such a wealth of topics is difficult, but I noticed one fact that seems remarkable. The better understanding of higher mental functions, and their mechanistic basis, that has come in the past decade through cybernetics has shown the outstanding importance of 'memory', both short- and long-term, in every aspect of the higher cerebral functions. Yet our knowledge of the physical basis of memory in the brain is to-day almost nothing. Our knowledge of brain function is grossly out of balance: of what happens from one milli-second to the next a great deal is known; but how a present event in the brain will influence its action in an hour's time, or in a day, or a year, is almost totally unknown. Yet it is the action that persists over a day or a year that is the really significant one for our everyday life. This lack of balance in our present-day science is shown in the book; for of the 152 contributors, only one specifically considers the effects of a drug on memory. But the same paper also shows that research in this direction is by no means forbiddingly difficult.

W. Ross Ashby

Life in the Sea

Text by Prof. Gösta Jägersten. Photography by Lennart Nilsson. Pp. 184. (London: G. T. Foulis and Co., Ltd., 1961.) 50s.

THIS is a book of photographs, a few in colour but mainly black-and-white, of marine invertebrates and a few fishes, taken by Lennart Nilsson. The text, and doubtless much scientific guidance, is supplied by Prof. Gösta Jägersten, of the Zoological Institute, Uppsala, and director of the University of Uppsala Biological Station, Klubban, Fiskebäckskil, on the Gullmar Fjord, where most of the photographs, apart from a few at Trondhjem, were taken.

It is much the best book of its kind I have seen. Apart from the high technical skill, especially of many of the photomicrographs, notably of protozoa, diatoms and a wide assortment of planktonic organisms, there is a remarkable quality of 'aliveness' about almost all the photographs; scarcely one is a set piece. Technical skill and artistic selection are blended with results which will come as a revelation of beauty as well as of biological information to professional zoologists no less than to amateurs and to school children. Perhaps the most striking pictures are those showing young pipe fishes emerging from the egg pouch of the male parent, the series (some in colour) showing conflict between an octopus (*Eledone*) and a lobster and another between two bristle worms, reared up with bared jaws. Of greatest scientific interest are the pictures of *Protodrilus* with Prof. Jägersten's description of the habitat of wave-washed sand and the effect of this on metamorphosis of the larvæ. Altogether a book for everyone and fully worth its price.

C. M. Yonge

Water Gardening

By Frances Perry. Third, revised edition. Pp. xviii+338+105 illustrations. (London: Country Life, Ltd., 1961.) 30s. net.

THE content of this book ranges far beyond the scope of the title, and the bulk of it consists of lists, somewhat arbitrarily defined, of plants which all supposedly have some relation to an aquatic environment. But the author's net has been cast much too widely, and the rather bewildering catalogue of species and forms could have been with advantage reduced to a judicious selection of plants which are readily obtainable, known to be of some horticultural merit and likely to be amenable to cultivation. Instead, it seems that the literature, not always the most up-to-date or authoritative, has been combed and many of the plants listed have little, apart from botanical interest, to commend them in the aquatic garden or its surroundings. The reader will find much of interest and profit in those chapters dealing with design, construction and planting of water gardens.

G. Taylor

SCIENCE AND GOVERNMENT IN A FREE SOCIETY*

By The Right Hon. VISCOUNT HAILSHAM, P.C.
Lord President of the Council and Minister for Science

IT is now a commonplace to say that in some respects a nation is highly regarded in proportion as its science and technology command respect. I do not, I think, have to prove this proposition. But I would wish to begin by qualifying it.

It is easy, of course, to demonstrate that the wealth of nations in peace, and their force and power of resistance in war, have largely been a function of their industrial and scientific strength. This is why all nations, slave and free, developed and undeveloped, do their best to achieve a high level of scientific and technological investment within their own borders. With most statesmen it is a simple question of the survival of their national societies, and the growth of their national wealth, that they should do so.

These considerations are obvious; what is not so obvious, and may, therefore, be in more need of argument, is that, at least in its higher reaches, science is not intrinsically a matter that should—or indeed can for long—be pursued solely for the sake of the wealth or the power that its pursuit may bring.

All genuine science has its origin in the questioning nature of the free human spirit, in its creative genius, in its power of insight. It is not something which can be dictated to either by theologians or ideologues—or, in the long run, by rulers. It may be that they can destroy it by repression, or even starve it for lack of material facilities. But they cannot by their own act make it live. It is doubtful how long they can even cause it to grow by supplying the means of growth. It is certain that they cannot, simply by supplying the means, dictate the nature or scope of the discoveries that can be made. Far less, indeed; because it is in the nature of scientific research to discover something which is intrinsically new. If anyone were able to predict, or pre-empt, the answers to scientific problems, they would not be genuine scientific problems, for the essence of the matter would already be known in advance. The discoveries of a Newton, an Einstein, a Darwin, a Planck, or a Rutherford are genuinely not known in advance, and, when they are made, their practical applications are not immediately perceived even by the inventors. This is something which lies deep at the root of the subject, and which statesmen and their people would do well to ponder more deeply than they do at present.

I say all this without for one instant losing sight of the spectacular achievements of science under the patronage of government, and without doubting for an instant that these achievements will be multiplied in our time—nor that a great deal of my own time as Minister must be spent precisely in seeking to multiply them in my own country. Nevertheless, there is a sense in which I continue to regard these wonderful achievements as something of a paradox, and I am tempted to speculate whether, in the end, the influence of a so obviously materially motivated interest by government in science will not obscure the very insights on which creative science is essentially based. In the U.S.S.R., and even perhaps in the United States, to look no nearer home, I see some signs that this may ultimately be so; if it does not prove to be the case it will be because the creative human genius in science, as in the arts, may prove too strong for the material motivation of the Governments which seek to direct its course.

However this may be, I begin by insisting that pure science is intrinsically as much a branch of culture as history, philosophy or poetry, and that the relationship between Government and pure science is essentially the same as that between an enlightened Government and the artist—that is, the relationship of an enlightened patron and not that of an employer. My point is that at its most vital and critical growing point, that of fundamental discovery, the Rutherfords and the Darwins have to be treated with not less respect than the Shakespeares and the Miltons and the Goethes. Historically, science is born of two highly respectable parents, the belief in the uniformity of Nature which was the natural child of monotheism in religion, and the freedom of expression and thought which was the product of the Renaissance. I would claim that if Government sought to interfere with the scientist in the absolute freedom of his explorations, and the integrity and independence of his speculation, it would ultimately destroy its real source of vigour, or alternatively frustrate its purpose by undermining the scientist's confidence and his will to co-operate.

But to this thesis there is a clear antithesis. The constant temptation on the part of Government to do this thing is matched by a constant need on the part of the scientist, especially in some ways the purest of the pure, to seek Government help on an ever-increasing scale. In most fields the day of the gifted amateur in science is rapidly closing. Some of the research tools of the modern scientist are very expensive. The education of the modern scientist is not only protracted and complicated, but almost as expensive as the tools he employs. He is increasingly dependent on the patronage, in some form, of Government.

But in exercising its patronage the Government is not, and cannot be, disinterested. The immense advantages which enure to a State that makes use of the discoveries of science—and trains its citizens to make them—are such that all States make some sort of an attempt to do so, and compete eagerly with one another in the attempt. Who will deny that, in the process, both the State and science have to some extent been corrupted? Three-quarters of British Government money spent on science come directly from the defence budget. The proportions in the United States and the U.S.S.R. are probably greater; indeed, it is questionable whether the basic organization of scientific endeavour in the U.S.S.R. makes any serious attempt to give civil science, as such, an independent life and existence at all. In Britain, the creation of a Minister for Science whose

* Substance of an address to the Arbeitsgemeinschaft für Forschung des Landes Nordrhein-Westfalen, Düsseldorf, on September 27.

terms of reference excluded defence was something of an act of faith, and has come in for some criticism. It is probably fair to admit that a price had to be paid for this. Nevertheless, I am still of the opinion that, in the long run, the marriage between science and defence is corrupting, and will at best turn science from a liberating to a destructive force, and at worst ultimately dry up the wells of inventiveness in the scientist himself. There is a point, indeed, at which technology itself will perhaps corrupt and destroy the scientific inventiveness on which it was based.

I therefore earnestly hope that at least a proportion of scientists in all countries will retain their freedom and integrity. It may be that the mere necessity to train all scientists in the genuine techniques of free speculation and invention will cause them to break their shackles. Certainly I am glad myself that my own position of Minister for Science is organized in such a way that neither defence nor even the short-term requirements of technology have been allowed to claim complete mastery over scientific endeavour.

But I would think that the strongest guarantee of a correct balance lies in a healthy relationship between Government and universities—not simply as regards the faculties of natural science, but generally in relation to their whole cultural purpose. There may be many means of making adequate government support available without infringing academic freedom. We are naturally proud in Britain of our own system, which allows government funds to be administered, in effect, by the universities themselves. I am not myself saying that this is the only possible way of achieving the desired object. But it remains, I think, at the basis of any healthy relationship of Government and science.

Indeed, whatever the temptation to militarize or commercialize science, there is a practical consideration which tends in the other direction. No country in the world has ever successfully set up a Department of Science, in the sense of a Ministry directly controlling the pace, the scope and the methods of scientific research. This is due to two considerations. The first is that the strategic planning of science cannot be undertaken without full participation of the scientists themselves—and by these I mean, not just a staff of administrators with scientific degrees, but also men and women who actually carry on scientific work, whether in universities, government research stations, or industry. This is as true of the socialist U.S.S.R. as it is of the capitalist United States, and it is not less true of Britain. Until a short while ago, Russian science was run by the Academy, and though the recent change by which it is now subordinated to a co-ordinating committee is almost certainly a move towards tighter control by the State, it is not a means of taking scientific decisions out of the hands of scientists, or reducing science to a mere branch of government administration.

There is also a theoretical argument which tends in the same direction. There is a sense in which there is no such thing as science, but only sciences. Another way of stating this is to say that science is in fact an all-embracing term, and that scientific researches into particular fields are functions of those fields and not of a comprehensive entity called science. From one point of view, medical research bears a much closer relation to the climate, population, health, diseases and economic activities of a nation than to their nuclear physics. In terms of science, as distinct from economic policy, it would be meaningless for a Finance Ministry official to try to block a grant for medical research on the ground that the money was needed for a synchrotron. It is true that both projects must take their stand in the queue for the general investment programme. But they are related to other items in the programme more closely than to one another.

What is wanted, therefore, is not a single scientific general staff forming part of a single department of science, but a series of scientific general staffs or Research Councils directing scientific research in the general fields to be occupied, independent of day-to-day bread-and-butter work in those fields, but in close contact with them. At the same time there must be, of course, some body to perform the more generalized function of supervision, looking for gaps or signs of imbalance, and fitting the parts into the general economic, cultural and social pattern of the entire community. This is the pattern of British organization. So, taking medical research again as an example, we have a Medical Research Council, independent alike of the Department of Scientific and Industrial Research and of the Ministry of Health, but under a Minister for Science, and of equivalent status to other Research Councils serving other scientific fields, commanding the respect and enjoying the co-operation of the scientists, but serving the public interest through its subordination to a Minister responsible for science, who, in turn, is advised by a general council on scientific policy.

The question arises, however, whether such an organization does not give rise to an ivory tower mentality on one hand, and lack of scientific-mindedness on the other. Critics have undoubtedly claimed that this is so, and it is sometimes said that on one hand British industry is too little conscious of the need for technological progress, and on the other hand, that the Research Councils are too strongly academic and too pre-occupied with fundamental research. I agree that the organization which I have been attempting to outline is not in any way a substitute for adequate technical or qualified staff at the executive and administrative level in industry or Government: indeed, I would go further, and say that it will only work at full efficiency on the assumption that such staff are available and active at every level, and in close contact with the Research Councils. This, however, does not diminish the case for the Research Councils, which is based on the need for detachment and independent supervision, and not on the supposition that they are substitutes for an appreciation of scientific and technical factors in those who are responsible for management in Government or industry.

This leads me to another generalization about the nature of my subject. Science is, or at least it should be, all-pervasive, and will not achieve its best results until this is the case. You can never make industry research-minded, whether privately or publicly owned, simply by providing research in government institutions or universities; and, if British experience is any guide, you will not even achieve it only by encouraging co-operative research by associations of individual firms—valuable though such associations be. There is no substitute, at any level, for science 'on the ground'—that is, within the individual industrial or commercial firm, within the individual teaching institution, within the individual government departments and nationalized corporations, and even within the home circle. This may seem impossibly

ambitious. But I consider it to be an obvious truth, whether you regard science as primarily a branch of culture or as something intrinsically practical in application. If the institution of a Ministry of, or for, Science were to obscure the essential truth that science and the scientific approach must be all-pervasive at every level, and cannot be imposed from outside, it would be nothing less than a national disaster.

Thus the duty of organizing science in a free society, like all other important duties, begins with the individual and covers the entire society. Government is a financing, co-ordinating, participating, training, function. Over a large field it is not, and cannot be, directory and executive. There are functions in which the main part ought to be played by industrialists, educationists, teachers and scientists themselves.

I now wish to discuss the conditions in which this duty can be carried out, and I begin, as anyone must begin, with the child. The clue to the correct organization of science is an adequate pattern of education. I would go so far as to say that if we could get this right, we should, in the long run, get everything right. If we get it wrong, we shall get nothing right, even in the short run.

By far the most important desideratum is the creation of a good general school education. You cannot make scientists of the first class out of an uneducated populace. The qualities which go to make a first-class scientist show themselves comparatively late in a child's career. In most disciplines a sound knowledge of mathematics is a condition of entry into the profession; in most of the others, such as medicine, a wide general culture is almost indispensable. Moreover, from every wider point of view, any attempt—perhaps in order to obtain rapid results, perhaps for short-sighted financial reasons, or possibly even for less-creditable reasons of political or ideological obscurantism—to generate a race of scientists interested only in their own specialities, to the exclusion of general culture or even of other scientific disciplines, would, I hope, be bound to fail. If it were not to fail, and if it were taken to anywhere near its logical conclusion, it would certainly be the most disastrous enterprise on which a developed human society could possibly embark. Such a race, narrow, blinkered, unbalanced, humourless, frustrated and—over a large range of human experience—inarticulate, would be one of the most evil that the human imagination could devise; probably a race of slaves designed to serve a race of still more uncultured masters; and, if not slaves, illiterate but technologically proficient tyrants to destroy or enslave the society which brought them into being.

To this first proposition, however, I would add an immediate footnote. In this sound general education of which I have spoken, science should form an integral part from the first. From the time that a child can read, and count up to ten, an element of scientific knowledge and thought should be injected into the curriculum of what he learns, even if it is only the most generalized statements about the universe, the microcosm of the atom, the organization of life and evolution, and the nature of chemical compounds. The reason why science is regarded by so many otherwise cultured people as a hidden mystery is because so little of it is injected into general reading and conversation before the age of ten [see also p. 387 of this issue]. We should not learn our culture—our history, our religion, our ethics, our physics, our mathematics—in a group of watertight vertically divided compartments, or in a series of horizontally divided instalments, but in a wide, integrated, smoothly flowing stream.

But behind the general educational pattern lies the need for a sound specialist and vocational training available from the age of fifteen onwards for those who need to know the essential manual skills or crafts, and from sixteen or seventeen onwards for those who seek technician status, or the higher technological or scientific degrees. I am not qualified to lay down a detailed or technical prescription for this. But I would say generally that there should be more than one road to the top, and that a society which does not make further education, of at least three years, universally available for those who possess the entrance qualification for it, on the basis that it will be availed of by at least 25 per cent of the appropriate age-groups in the population, is seriously under-estimating its requirement. If you put the figure at 40 per cent I would not seriously quarrel, but would say that this remains to be proved.

At the spearhead of the scientific movement is the army of research workers, the true frontiersmen of scientific advance, few in numbers, highly specialized in qualification, but on whose efforts depend the success or failure of all. How will these work? Who will direct their work? How will it be financed and organized? What is to be their relationship to Government? These questions are really at the heart of the whole matter; everything else is, in comparison, only on the fringe.

In the early days of science, these questions presented no difficulty. Science was independent of Government and even, to a large extent, of the formal courses of the university. But this is no longer the case. The men of whom I now speak take about thirty years to train—five in the nursery, ten in the school, three or four in the university as undergraduates, four or more learning research methods, and perhaps another five working under some kind of supervision. Their research apparatus—originally made of glass and brass in an attic or a garage or outhouse—may now cost millions of pounds, like the proton synchrontron at CERN, or the fast breeder reactor at Dounreay; and the supporting structure of constructional engineering, electronics, computers, served by technicians and craftsmen, employs whole industries and forms the basis of life in whole townships and communities. Of those who start off on this road, many fall by the wayside. On the genius of a small proportion out of the very few who survive the race depends the success or failure of all.

Here my first proposition is that, at the highest level, it is ruinous to separate research and teaching. It follows that the most advanced scientific work should be located in or near the universities, where this is possible, or directed by university personnel. The contrary course—the creation of separate government-sponsored institutes—is attractive and is sometimes rewarded with spectacular results. Moreover, considerations of national security may sometimes dictate it. But in the end it can be a primrose path, as everyone from the U.S.S.R. westwards who has tried it knows. You may gather together all the most promising intellects in a subject, build them a town, equip them with all the most lavish facilities, and the thing will go off like a rocket—at first. But after five years—after ten, after fifteen—what then?

Unless active steps are taken to redress it, there is a danger that intellects will be staler, new blood will not be forthcoming, the stimulus of youth will be absent. From a proportion of those engaged, the research potential may have disappeared; even the scientific questions will have changed. Instead of a research institute you would then have a monastery which had lost its faith—isolated, stereotyped, remorselessly carrying on activities with a diminishing margin of useful results.

There have been, of course, remarkable examples of research institutes where the dangers I have mentioned have been avoided, it would seem, indefinitely. In Great Britain I would cite the National Institute of Medical Research and the National Physical Laboratory, and in Germany the Institutes of the Max Planck Society. But if the dangers have been avoided, it is precisely because they have been recognized as dangers. As a general proposition I adhere to the view that there must be the constant stimulus of teaching, or research will tend to die. There must be the constant interpenetration of the mature intellect with the adolescent, or it may petrify. There must be a constant outlet for research workers who are ready to pass into teaching or administration, or research may even degenerate into a racket. Therefore, where possible, locate your best research in university or teaching institutions. If you cannot, you must do the next best thing and arrange for constant interchange of personnel, and regular exchange of ideas between them.

This does not mean that there is no place for government research institutions, or institutions of research collectively organized by or for the benefit of private industry. On the contrary, as will be seen, my thesis is that there is room for more and more of these. My point is only this: that however much these bodies proliferate, they should where possible locate their best research in or near universities, and that where they cannot do so they should promote the closest possible links with teaching institutions of all sorts.

To begin, therefore, with the Government. Most governments—directly or indirectly—build the roads, manage the telephones, dispose of the sewage, construct and maintain the harbours; many manage the railways, generate the electricity and gas. All are concerned with standards of measurement, heat, light, health and air pollution. Most are concerned with the nutrition and health of their citizens, and few can divorce themselves from the efficiency of agriculture. All these activities throw up problems of research and development, many of which cannot conveniently be carried on in conjunction with teaching. Some activities are intrinsically public in character. Others can efficiently be financed only by heavy drafts of public money. It will be a long time before agricultural research is paid for entirely by farmers. Nor can the pharmaceutical industry, the medical profession, or private generosity, finance medical research on an adequate scale. Even in the sphere of industrial research there are many general questions, like those connected with fatigue and creep in metals, which lend themselves to government research financed by Government, because they are not so closely connected with any particular private industry as to attract sufficient private finance.

Here I would describe in greater detail a British institution which we owe in part to the genius and foresight of the first Lord Haldane, and which has on the whole won the confidence of the scientific world and created the means of providing government finance without sacrificing either academic independence or scientific integrity. This is the institution of the Research Councils, of which there are at present four in Britain; the Department of Scientific and Industrial Research, the Medical Research Council, the Agricultural Research Council, and the Nature Conservancy.

The thinking behind the creation of these Councils is to separate the activity of research from the executive business of government and economic activity—keeping medical research separate from the Ministry of Health, for example—and to concentrate it under a number of Councils, composed mainly of working scientists and industrialists with real executive responsibility for the research programmes under their control, with separate budgets negotiated with the Finance Department, and invested with the responsibility, within their several spheres, for financing the training of research workers no less than carrying forward the business of research. All operate stations or institutes of their own. All finance research in the universities; and one, the Department of Scientific and Industrial Research, co-operates with particular industries in financing joint programmes of research carried out by organizations known as Research Associations, managed primarily by the industries concerned on a co-operative basis, but attracting also government grants through the Research Council.

It would, of course, be a logical and attractive arrangement if one could go on to report that the fundamental or basic research in Britain were now carried out in teaching institutions, and that the applied research was all done in government or industrially based research institutions.

Experience, however, shows that, although this may in theory and in practice approximate to the ideal arrangement, no absolute division of function on these lines is either desirable or possible. Apart from the obvious fact that any line drawn between basic and applied research is necessarily arbitrary and imprecise, experience shows that the human problems involved cannot be solved satisfactorily on these lines. However closely applied or related to concrete industrial difficulties the work of a research institution may be in intention, in practice it will not retain the services of high-grade scientific personnel if they are to be completely debarred from undertaking fundamental research.

At the other end of the scale, no matter how academic may be the work in a university, it is impossible to exclude applied or practical applications or development. Contact with the intellectual frontier is a requisite for the mental health and happiness of one, contact with real life an unavoidable necessity for the other. Thus no division is absolute, and nothing will substitute for the management of these highly sensitive, highly qualified human beings by suitably qualified and sympathetic scientific bodies assembled for the purpose. A close association between basic and applied science, and a constant interchange of personal contacts between industrial and governmental research, and between both and the teaching institutions, are probably a better prescription than a more perfectly formalized pattern of organization.

How far is it possible for national Governments and administrations to guide and fertilize science

without warping its character and distorting its scope? I have already pointed out that the gifted amateur, and even the rich private or industrial patron, whether individual or corporate, cannot possibly cover the sweep of modern science and technology, or even the training of the modern scientist; the needs have reached the national scale.

But the nation State also has its limitations, even financially, and more still in its terms of reference. It is surely no coincidence that, in one field after another, science not just on a national but also on an international scale is beginning to become an actuality. The International Geophysical Year is only one example—in which even East and West co-operated. Europe has recently begun to plan to enter co-operatively into space research. Britain and the United States are co-operating in the launching of the first British satellite experiments. Western European countries co-operate, under the auspices of the European Nuclear Energy Agency, in the *Dragon* High Temperature Reactor project at Winfrith Heath, and in the Halden project in Norway. I have already spoken of the internationally owned proton synchrotron at CERN. There are projects on foot for both European and Atlantic universities. Much of the aid to under-developed countries will be both co-operative and technical. What is the future and what are the prospects for enterprises of this kind?

I would say that a very considerable scope exists for international scientific effort. In the long run, I am quite certain that the really ambitious space research, if it is to continue indefinitely, will have to be financed in this way. I cannot myself conceive that either medical or agricultural problems would fail to gain by international treatment. Many of the longer-term projects in engineering, for example, in the realm of thermonuclear fusion or the direct generation of electrical energy from heat, may well come into the same category.

At the same time I would emphasize that international co-operation is no substitute for national excellence. Our experience has been that co-operation is only possible between nations which each have something comparable to offer to one another. It is neither practicable nor desirable to create international scientific organizations to make up for a basic want of scientific organization or effort in the individual member countries. The new organizations, when they come into being, will be additions to and not substitutions for what is attempted nationally. They will be no excuse for the individual member States to neglect either national education or research on a national scale—although duplication of effort can be avoided, and a scale effect obtained by jointly undertaking projects which are in excess of the capital resources available to any one.

Hitherto it has been possible for the nation State to stimulate the growth of scientific effort in a variety of different ways—by supporting scientists in the universities or research institutions, and financing their machines and apparatus, and by developing machines capable of practical application in various directions where the State itself is the ultimate user of the machine.

But this has meant, in societies both free and servile, predominantly the development of engines of war, even where these have ensuing or concurrent civilian application. Where would the aeroplane, the radio, radar, jet propulsion, or, more recently, space research have been but for the constant urge of governments to prevail in two world wars—or should I say in three? It is useless for governments to protest that their Gagarins and their Sheppards—or their Titovs—have also achieved technical success in realms of peaceful science and technology. Only those nations which have had reason to develop military rockets have actually reached for the stars, and a principal effect of their space flights has been to emphasize the prestige of their military technologies.

Is there no means nowadays of governments developing or helping to develop new devices which are just as revolutionary but without the stimulus of war or the fear of it? The steam engine in its origin, and the internal combustion engine in its inception, were primarily civilian devices. What must we do now to stimulate development in a purely civilian sense?

I must begin by emphasizing that, as between research and development, it is development which costs the money. Of the two hundred and fifty million pounds spent on research and development by government departments in Britain, about fifty million goes on research and the rest, I estimate, on development, and of this, of course, a proportion that I would not care to guess at is spent on projects which never ultimately reach fruition.

Secondly, I point out that the role of the would-be financier of development is radically different according to whether the financier is, or is not, the ultimate user of the hardware to be produced. One reason why so much successful development has been inspired by defence has not been the over-riding importance of the conception of war in the mentality of modern government; it has been the simple circumstance that the defence forces as the ultimate users of the hardware have been able to formulate their staff requirements, undertake their feasibility studies, and finally proceed to development contracts with the makers, without the discipline of the necessity to make a commercial project, but with all the enthusiasm of a potential user whose life may depend on the excellence of the product.

It is not easy, in a free society, to reproduce all these conditions in civilian life—even where, as is by no means usually the case, the Government is the ultimate consumer. In civil aircraft, in railway engineering, in road construction, radio engineering, electricity generation, perhaps in machine tools and computers, and above all in the peaceful uses of atomic energy, some of the conditions exist, and only in the latter case have the possibilities been at all adequately grasped—with results which have as yet not been fully seen. Admittedly there are formidable difficulties—the formulation of the requirement, the provision of government finance for a project which may ultimately enure at least in part to private profit, the placing of a contract selectively (which could give ground for scandal and abuse); but we shall not advance farther until we get our feet wet. It may be that here, too, there is scope for the pooling of resources internationally.

Of one thing I am quite certain. The preservation of a technological lead cannot long be maintained by means of commercial secrecy. Commercial, like military, security always breaks down in the end, and what advantage exists in priority is obtained by industrial experience in production rather than by the maintenance of technical secrets.

A nation remains in the forefront technically, not by patenting its devices but by keeping ahead in invention and development, and this is a complex achievement which lies only in part within the province or power of government to promote. Let us remember that science is an achievement of the human spirit, and resolve that the European peoples will foster it for its own sake rather than for the wealth and power that it will bring. The respect for truth, the freedom of intellect, the moral discipline that it engenders, are worth more than these. Get them for their own sake, and my conviction is that what remains will be added unto us.

SYSTEMATIC MEASUREMENT OF FALL-OUT IN CZECHOSLOVAKIA UP TO 1961

By Prof. W. SANTHOLZER, G. MACKU and J. PODZIMEK

Institute of Medical Physics, Charles University Medical Faculty, Hradec Králové, Czechoslovakia

SYSTEMATIC measurements of fall-out in Norway[1] showed that monthly average concentration of fall-out materials in precipitation after high-yield test explosions reaches a peak value in about six months, and then decreases with a half-life of about one month. Our systematic measurements of fall-out in precipitation in Hradec Králové, Czechoslovakia, during the past four to five years provided evidence that the activity of radioactive fission products from fall-out during November 1956–December 1959 showed a trend similar to that in Norway[2] (Fig. 1). The peak of the activity in April 1959 has about the same value, 3,960 μμc./l. precipitation. The minimal value during November–December 1959 was about 20 μμc./l.

Detection of French Nuclear Tests of 1960

In Fig. 1 are shown the results of our measurements also during January 1960–March 1961. The increase in activity of rain-water in March 1960 after the first French nuclear test in the Sahara Desert on February 13, 1960, was very pronounced but transitory. Activity of fission products reached its highest level in the collection of rain-water of March 1, namely, 25·9 mμc./l. In the collections of March 4–12 the activity was about 5 mμc./l. The concentration of radioactive fission products from fall-out in the air at ground-level in Hradec Králové during March 1960, studied by measurements made on dust collected by a membrane filter assembly, showed no pronounced increase in activity. (The concentration was about 1 μμc./m.³.) It is interesting to note the measurements of Patterson and Lockhart on the eighth meridian network of air-filter stations[3]. The activity of fission products reached its highest level at Miraflores, Panama, in the collection of February 26–29, and this station received the first indication of fission products in the 2-day collection of February 24–26. It is therefore probable that the radioactive cloud from the first French nuclear test reached our station in Hradec Králové at the beginning of its second revolution around the globe, its velocity of propagation being about 100 km./hr. The attempt to explain the relatively high level of the activity of rain-water and the simultaneously low level of the activity of surface air sampled on March 1 in Hradec Králové is made in the last paragraph. A sharp increase in the level of activity occurring some time during February 28–March 1, 1960, in Surrey, England, and evidence which suggests that this was due to the first nuclear weapon test in the Sahara, was described by W. Anderson *et al.*[4].

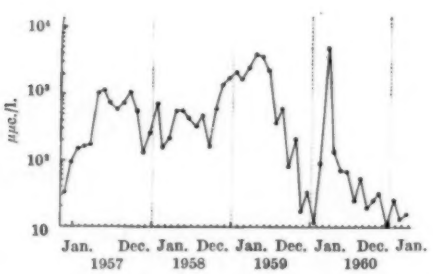


Fig. 1. Monthly average concentrations of materials from fall-out in precipitation

Fig. 2 shows the decay of three representative samples plotted on a log activity versus log time-scale with February 13 as the origin. Fig. 2 is similar to the figure published by Y. Feige *et al.* of the Israel Fall-out Monitoring Service[5], where the main impact of the first French nuclear explosion was already on February 17. Our Fig. 2 demonstrates that the exponent n in the formula of the decay $A = kt^{-n}$ is 1·2–1·4. The long-lived background becomes evident after the elapse of 120–160 days, according to the level of the activity of fresh samples. We followed the decay during 400 days.

After the second French nuclear test, on April 1, 1960, we picked up radioactive materials in rain-water on April 9. The initial activity was relatively low, about 0·4 mμc./l., and average long-lived background activity had to be subtracted after 30 days.

Patterson and Lockhart[3] relate the second rise of the activity during early April to the February test, since debris from the French test of April 1 would not be expected to arrive so soon, and debris from the much smaller second test could be hidden by residual activity from the first test. If we relate at our sampling station the second rise during early April to the first French test, the exponent n in the formula $A = kt^{-n}$ would be 3–4, which is unlikely. The first 'half-life' of our sample collected on April 9 was 9–10 days, which is probably due to the fresh activity.

In Rumania, Mageru *et al.* observed the transitory increase in activity during February 23–25 and March 16–20, 1960, in the air-streaming from the Mediterranean[6].

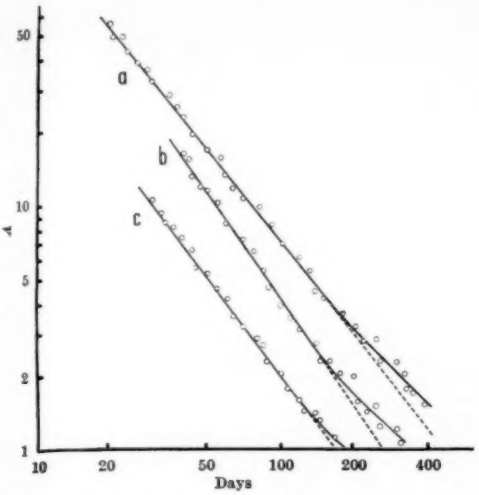


Fig. 2. The decay of three representative samples in the collection of March 1–8, 1960, after the first French nuclear test on February 13, 1960

Autoradiograms of radioactive dust particles from the second French test were obtained by V. Havlovic in Pilsen, Czechoslovakia[7].

After the third French test on December 27, 1960, we did not observe any marked increase in activity.

Collection of Fall-out and Typical Decay Curves

In addition to the collection of rain-water samples, the fall-out was collected every second or third day by the pot method, which is efficient[8]. The collecting dishes, made of polyvinylchloride, have high borders and a collecting area of 1 m.². A thin layer of distilled water is maintained on the bottom. For control purposes the fall-out is also collected once a month. After annealing at a temperature of about 600° C., the sample is again moistened and uniformly applied to an aluminium dish, 3 cm.² in area. After drying, and three days after collection, the activity is measured with a Geiger-counter for beta-rays. The number of pulses is recorded with automatic equipment of our own construction on a paper tape[9]. The samples are also changed automatically. Strontium-90 + yttrium-90, obtained from the Dosimetric Department of the Institute of Nuclear Research in Prague, was used as a standard.

Fig. 3 shows the activity of fall-out in the period when nuclear tests ceased during November 1, 1958–February 29, 1960, and also during March 1, 1960–March 31, 1961. Activities are in mc./km.²/month, and plotted as logarithm of activity versus time. In Fig. 3 are used activities collected monthly, that is, at the end of each month. With this value we compared the total of daily activities for the month considering the radioactive decay. The maximum in March 1960 is not so pronounced as in Fig. 1, because the fresh activity in March 1960 decayed more quickly than relatively older activities at the beginning of 1959. In the first quarter of 1961 the monthly average activity of fall-out was 0·4 mc./km.².

Fig. 4 shows the decrease with time of activity of rain-water collected on May 4, 1959 (sample *a*), and on March 1, 1961 (sample *b*). Approximate date of nuclear test was determined graphically. The values of :

$$1 : {}^{1.2}\sqrt{A} \text{ and } 1 : {}^{1.6}\sqrt{A}$$

are plotted on the ordinate axis, thus producing curves *a'*, *a''* and *b'*. (The curve *b''* is not plotted.) In the period of the first 150 days they are straight lines, which intersect the time-axis in July, and October 1958 and in February 1960. For the first French test one may plot the values of $1 : {}^{1.2}\sqrt{A}$ to obtain the right date of the test. For the sample collected on May 4, 1959, which was one of our last relatively strong active rain-water samples in the spring of 1959, it is impossible to determine by using the rather insensitive graphical method which date of the nuclear test is correct[8]. But a control graphical determination makes some date in October 1958 more probable. It consists in the fact that for the relation $A = kt^{-n}$ there is a linear dependence between the time difference (τ) of the dates corresponding to the activities, the ratio of which was chosen constant, for example, $A_n : A_{n+1} = 2$, and the time (T) corresponding to the first activity. Relation $A = kt^{-n}$ may be written in the form $A = a\,(T - T_0)^{-n}$, where T is the date of measurement, T_0 the date of the nuclear test, and then transformed in the linear dependence $\tau = c\,(T - T_0)$, where the constant c is dependent on the exponent n, $c = {}^{n}\sqrt{2} - 1$. Thus, $n = 1{\cdot}2$ appears more probable (curve *a'*).

In fresh samples collected during March 1–14 two groups of beta-rays were roughly determined by absorption measurements with aluminium foils: 0·7 MeV. and 1·5 – 2·1 MeV. In agreement with Akpinar[10], it was shown that the energy of beta-rays

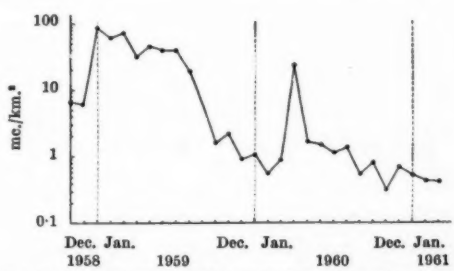


Fig. 3. Concentrations of fall-out materials in mc./km.²/month

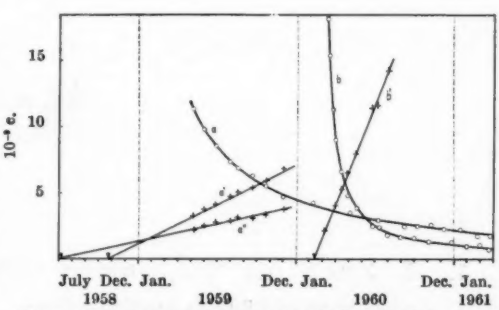


Fig. 4. Typical curves of decrease in activity with time of two samples of rain-water collected on May 4, 1959 (*a*), and on March 1, 1960 (*b*). Approximate dates of nuclear tests were determined graphically from the initial part of the curves, where the background is negligible

varies with the age of the sample, but due to relatively large masses of samples we were unable to use this method for rapidly determining the age. Old samples collected in spring 1959 and studied by absorption measurements showed the group of beta-rays about 3 MeV.

The energy of the gamma-rays of samples in the collection during spring and summer 1959 was roughly determined from absorption measurements with copper and lead plates, with a scintillation counter. The result is 0·2 MeV. and 0·8 MeV. This is in rough agreement with gamma-scintillation spectra of the fall-out[4,11], and with the main gamma-emitting nuclides to be expected in a 6–18 month-old mixture of fission products[12].

The seasonal variation of the first half-life[13] of our samples corresponds roughly to the calendar of nuclear test explosions, as was more exactly determined by D. H. Peirson *et al.*[14] on the basis of the ratio of zirconium-95/cæsium-137, which is a useful index of age or origin of the mixture of fission products.

Radiostrontium determinations in the fall-out were made using well-established methods[15]. (Separation was carried out by the Chemical Institute of the Medical Faculty of the Charles University in Hradec Králové.) The average concentration of strontium-90 found by us in rain-water collected during the first two quarters of 1959 was about 10 μμc./l., in the last quarter about 1 μμc./l., and in the first quarter of 1960 about the same. This is in agreement with the measurements of W. Anderson *et al.*[12], who proved a marked reduction of the average quarterly concentrations of strontium-90 in rain-water collected during 1959.

Correlation with Meteorological Data

The reality of the maximum of man-made radioactivity in rain-water measured on the territory of Č.S.S.R. at the beginning of March 1960 is confirmed by comparison with the meteorological situation. A picture of the upper air flow at the 500-mb. level has shown that the contaminated air mass after the first Sahara test reached our country in 17 days. During this time the trajectory of the air mass could be traced over northern Egypt, Iran, northern India, Vietnam, southern China, the Pacific, the southern parts of the United States (February 26), the Atlantic Ocean and northern France. It is characterized by the very rapid progress, especially in the part above the Atlantic Ocean, where the velocities in the jet-stream surpassed 100 km./hr. The trajectory explains the measured maxima of the radioactivity in air samples in Israel on February 17, a sharp increase of the activity in Surrey, England, on February 29 and on March 1 in Germany. On an average, however, following the trajectory, we find the maxima of the air activity above the territory of Israel on February 15 or 16. From this we arrive at the hypothesis that a radioactive cloud settled relatively slowly and that the air trajectory in the lower levels was probably not the same as in the 500-mb. level.

The abnormally high level of artificial radioactivity of atmospheric precipitation on March 1 does not correspond in Hradec Králové to the maximum, which is not expressed so well in the histogram of air activities. We explain this fact by the process during which radioactive particles were bound in clouds and washed out below the clouds by the falling precipitation elements. In this case a decisive part is played by the evaporation of rain-drops. As the intensity of evaporation increases with decreasing dimension of drops, we could expect, at 11 hr. on March 1, when the cold front with smaller intensity of precipitation and smaller dimensions of rain-drops passed over our country, a higher radioactivity of rain-water, although the measured values of air activity was relatively low.

We are indebted to Prof. F. Běhounek, member of the Czechoslovak Academy of Sciences, for valuable advice and encouragement during this work.

[1] Hvinden, T., Hveding, G., Lillegraven, A., and Small, S. H., *Nature*, **185**, 805 (1960).
[2] Santholzer, W., *Czech. J. Phys.*, B **10**, 595 (1960). Santholzer, W., Podzimek, J., and Macku, G., *Studia geoph. et geod.*, **2**, 353 (1958). Macku, G., *Automatisierung Zmsr.*, **3**, 395 (1960).
[3] Patterson, R. L., and Lockhart, L. B., *Science*, **132**, 474 (1960).
[4] Anderson, W., Bentley, R. E., Burton, L. K., and Greatorex, C. A., *Nature*, **186**, 223 (1960).
[5] Feige, Y., Shalmon, E., Vardi, J., Gat, J. R., and Tokatly, Y. L., *Nature*, **189**, 90 (1961).
[6] Mageru, V., Bianariu, D., and Gabe, I., *Naturw.*, **47**, 319 (1960).
[7] Havlovic, V., *Nature*, **189**, 977 (1961).
[8] Shvedov, V. P., Blinov, V. A., Gedeonov, L. I., and Ankudinov, E. P., *Atomnaya energiya*, **5**, 577 (1958).
[9] Franke, T., *Atomkernenergie*, **4**, 308 (1959).
[10] Akpinar, S., *Nucleonics*, **15**, 88 (1957).
[11] Peirson, D. H., and Salmon, L., *Nature*, **184**, 1678 (1959).
[12] Anderson, W., Bentley, R. E., Burton, L. K., Crookall, J. O., and Greatorex, C. A., *Nature*, **186**, 925 (1960).
[13] Hinzpeter, M., Becker, F., and Reifferscheid, H., *Strahlenschutz*, No. 7 (1959).
[14] Peirson, D. H., Crooks, R. N., and Fisher, E. M. R., *Nature*, **186**, 224 (1960).
[15] Kool, J., *Analyt. Chem.*, **30**, 532 (1958).

RADIOACTIVE FALL-OUT AND THE TESTING OF NUCLEAR WEAPONS

STATEMENT BY THE MEDICAL RESEARCH COUNCIL

In December 1960 the Medical Research Council published its second report "The Hazards to Man of Nuclear and Allied Radiations" (Cmnd. 1225), which included an assessment of the levels of exposure to radiation which had resulted from test explosions up to that time. The resumption of nuclear weapon testing in the atmosphere by the U.S.S.R. has, however, resulted in the further release of radioactive fission products and, at the request of the Government, the Council has issued a statement (October 24) which attempts to put in perspective some of the more important considerations that result from the new situation. A review of this subject would not have been possible without help from many people, and the Council would like to record in particular its indebtedness to the Agricultural Research Council and the United Kingdom Atomic Energy Authority, members of the staff of which have provided valuable advice and assistance.

Mechanism of deposition of fall-out. Work on the deposition of fall-out from weapons detonated during 1958 and in previous years has shown that the rate at

which fission products pass from the stratosphere to the lower atmosphere and thence to the Earth's surface varies greatly according to the size of the explosion and the height, latitude and time of year at which it occurs. Deposition from large explosions has been found to be most rapid when debris is released into the lower stratosphere in high latitudes during the autumn.

Types and sources of radiation exposure from fall-out. The significance of tests involving substantial and rapid fall-out is that, soon after the tests, considerable quantities of short-lived radioisotopes, which will not have been retained in the stratosphere sufficiently long to decay to negligible levels of radioactivity, are likely to be deposited on the Earth's surface. These short-lived components of fall-out contain both materials, such as zirconium-95, which are not absorbed into the body but contribute to the external radiation received by the whole body, and materials which are absorbed into the body such as iodine-131, which becomes concentrated in the thyroid gland. If present in sufficient amounts, these short-lived materials may, in the short time during which they retain their radioactivity, give doses comparable with those delivered over much longer periods by the longer-lived materials produced in the same explosions.

The chief longer-lived components of fall-out which require consideration are cæsium-137, carbon-14 and strontium-90. Cæsium-137 and carbon-14 are of importance because they give rise to general irradiation of the body, including the gonads. The dose-rate from carbon-14 is very much lower than that from cæsium-137 but, as it will continue for very long periods, the total dose over hundreds of generations may be similar. Strontium-90, both because of its long half-life and because it becomes concentrated in bone, causing prolonged irradiation of bone and bone marrow, is still regarded as the most important single indicator of the possible total hazard to the individual.

Assessment of the hazard from fall-out prior to the present tests. The Council's second report on "The Hazards to Man of Nuclear and Allied Radiations" (Cmnd. 1225), which assessed the possible extent of the hazard caused by test explosions up to the end of 1958, provides some background for considering the possible consequences of recent tests.

With regard to the genetic effect from all test explosions which had taken place up to the end of 1958, the Council concluded that the mutations which might be caused were likely to be few in relation to those produced by natural radiation: the latter are themselves believed to form one-tenth or less of the mutations newly arising in any generation.

As to effects on individuals, among the most important components in fall-out are iodine-131 and strontium-90. From the amounts of iodine-131 observed in human thyroid glands, the Council concluded that the average thyroid dose delivered by this radioisotope as a result of nuclear explosions which had taken place prior to its 1960 report was unlikely to have exceeded one-quarter of the radiation received from natural sources during any year.

Investigations of strontium-90 in human diet in the United Kingdom were commenced in 1955 and were considerably expanded in 1958. The contamination of milk receives particular attention because dairy produce contains most of the strontium-90 which the population ingests. Samples of milk drawn from depots handling more than 40 per cent of total production are examined. Other foods are also sampled, so that the average strontium-90 content of diet throughout Great Britain can be assessed.

Between 1958 and 1959, the ratio of strontium-90 to calcium in average diet in the United Kingdom increased by 50 per cent, but the recent Agricultural Research Council report (*ARCRL 5*) shows that in 1960 it decreased to little above the 1958-level. This decrease was due to the reduced rate of fall-out consequent on the cessation of testing and reflected the fact that, when the radioactive fall-out was relatively high, the contamination of diet was due primarily to the direct entrapment of the recent deposit on growing vegetation. Strontium-90, however, also enters plants from the soil. This is a slower but continuing process so that, even if fall-out ceased, diet would continue to be contaminated, though at a reduced level, for a considerable period.

The results of assays of human bone are now available for the five years 1956–60. In each year the highest concentration of strontium-90 was found in children about one year old. In this age-group there was a progressive but slow rise each year until 1958, a doubling of the 1958 value during 1959, but a fall in 1960 which has continued in 1961. The average value in these infants of 4 strontium units (that is, 4 $\mu\mu$c. per gm. of calcium) at the peak in 1959 was about a sixteenth of the value (67 strontium units) regarded by the Council as the maximum permissible average value in the whole population.

Children born in the year 1955 have been exposed to strontium-90 from their diet during their whole life-time. Although in 1956 the average value in their bones was 1 strontium unit, the value in 1960 was only fractionally greater (1·4 units). Older children have formed only part of their bone structure from contaminated food, and the bones of those born between 1945 and 1950 showed only 1 strontium unit in 1960.

Estimation of additional exposure due to recent atmospheric tests. The recent tests by the U.S.S.R. are reported to have included explosions both in the kiloton- and the megaton-range and to have occurred in Central Siberia and the Arctic. As would have been expected from experience in 1958, the radioactivity reaching the ground in recent weeks as a result of the present series of explosions has come mainly from short-lived components which are deposited within a few weeks of the explosion. The deposition of longer-lived components, such as strontium-90 and cæsium-137, is likely to be greatest next spring.

Since iodine-131 is the main short-lived isotope absorbed into the body, extensive measurements are being made of levels of iodine-131 contamination following the present series of tests. Early results of the survey, which is being carried out by the Agricultural Research Council, are given in the appendix.

It is estimated that the average radiation exposure to the thyroid from this isotope in the fall-out already measured in the United Kingdom from the present tests is likely to be less than the figure of one-quarter of the radiation received from natural sources during any year. However, the value for infants in their first year of life may now approximate to that received annually from exposure to natural sources.

Because iodine-131 is taken into the body predominantly through milk, the Medical Research Council has had under review the maximum sustained

level in milk which might be regarded as allowable. It has based its recommendations on similar criteria to those used in setting the dose-levels permissible in the general population from strontium-90. It has concluded that an acceptable dose would not be exceeded in any age-group of the population unless an average concentration of iodine-131 in milk of 130 μμc. per litre was exceeded over a period of one year, or higher concentrations for correspondingly shorter times. The level has been set to provide a margin of safety for the section of the population in the group in which the dose-rate would be highest, namely, infants up to the age of 1 year; considerably lower radiation doses would be received by older children and adults consuming the same milk. Allowance has also been made for the likely variation in the concentration of radioiodine in local milk supplies. The dose referred to will not be reached unless present levels of iodine-131 continue for several months as a result of further weapon tests or are substantially increased. A marked decrease may be expected within a few weeks of the cessation of tests.

As the highest fall-out rate of some of the longer-lived radioactive substances, such as strontium-90, may be expected in the late spring of 1962, it is not yet possible to predict the total additional radiation dose to which the population has been committed by the recent tests. However, as the levels of radioactivity measured in air have been similar to those observed at an equivalent time after explosions in the autumn of 1958, there is at present no reason for believing that tests from which fall-out has so far been detected will involve an additional radiation exposure from external radiation or from strontium-90 which differs greatly from what has been experienced hitherto. It may be concluded that strontium-90 from tests so far carried out will not give rise to levels in bone approaching those which the Council laid down in its report of December 1960 as the maximum permissible in the general population.

Consideration has also been given to the inhalation of radioactive materials, including plutonium. It has been concluded that the extent of radiation exposure from such sources is unlikely to be comparable in importance with that from the other sources mentioned.

The Council is informed that it is not expected that underground tests, such as the United States has been carrying out, will contribute to world-wide contamination.

Possible consequences of the explosion of a fifty-megaton bomb. It appears likely that the explosion of a fifty-megaton bomb during the present series of tests would cause the total energy released from this series to correspond approximately with that of explosions occurring in the two years 1957–58 (according to the Ministry of Defence statement of May 5, 1959). If the bomb is exploded in the atmosphere, the resulting fall-out will depend on the extent to which the energy is due to fusion rather than fission, and on the altitude and site of the explosion.

The explosion of such a bomb in the atmosphere in northern latitudes during the autumn could be followed by considerable fall-out of shorter-lived radioisotopes. Deposition of radioiodine due to the series might then be substantially greater than that from previous tests, and the contamination of milk in the subsequent weeks might cause the annual mean level to approach that already specified, namely, 130 μμc. per litre.

Radioisotopes of medium half-lives, such as zirconium-95, might be expected to raise the level of external radiation in the open for some months by an amount comparable with the dose-rate received from natural sources.

It is not yet possible to predict with any accuracy the total additional dose of radiation to which the population will be committed by the current series of Russian tests, including the explosion of a fifty-megaton bomb. Concentrations of strontium-90 in bone in young children might rise in the course of one year to about twice the maximum level observed hitherto, but the level regarded as the maximum permissible one by the Medical Research Council would not be reached.

Arrangements for monitoring and assessing the fall-out situation. Regular measurements of radioactivity in rain, drinking-water, food (including milk), air, soil, vegetation and human bone, are continuing according to a country-wide programme under the supervision of the Medical Research Council and the Agricultural Research Council. The results of measurement of radioactive fall-out are published at frequent intervals. Premature attempts to interpret the significance of individual early measurements directly in terms of human hazard can be extremely misleading. Variations in the measurements are to be expected, both from day to day and as between one place and another. The significant factors are average levels over long periods of time.

The situation with regard to fall-out and the hazards from it is kept under close and continuous review, and the bodies responsible for so doing have undertaken immediately to inform the Government if there should be any major change in their assessments.

Appendix: Measurements of Iodine-131 in Milk

When it became apparent that the new series of nuclear explosions might lead to appreciable quantities of iodine-131 in milk, the Agricultural Research Council Radiobiological Laboratory commenced an examination of milk from eight depots or farms located in central and southern England, Wales, Scotland and Northern Ireland. The samples are analysed frequently, usually daily. The mean results so far obtained are shown in Table 1.

In the first few days of the week commencing October 15, 1961, the average level fell to below 100, but those results which are now available for the

Table 1. Levels of Iodine-131 in Milk Produced at Eight Sites in Different Parts of the United Kingdom

Week commencing:	μμc. Iodine-131/litre
September 16, 1961	85
September 24, 1961	109
October 1, 1961	138
October 8, 1961	150
Mean for four weeks	120

Table 2. Levels of Iodine-131 in Milk Produced Throughout the United Kingdom

Zone	Mean of values for October 2 and 16, 1961 μμc. Iodine-131/litre
1. Northern England	103
2. North-West England	92
3. East and East Midland	71
4. West Midland	100
5. Wales	153
6. South and South-East England	102
7. Mid-Western England	86
8. Far Western England	105
9. Scotland	190
10. Northern Ireland	165
Country-wide mean weighted for production	113

latter part of that week indicate a return to the levels observed ten days previously.

More detailed investigations of the general level in milk throughout the country were made for October 1 and 15. On these dates representative samples were obtained from depots which together handle more than 40 per cent of the total milk produced in each of ten zones into which the whole country has been divided. The statistical organization of this elaborate scheme, which is also in operation for strontium-90, has been described in reports by the Laboratory. The average results for the two sampling occasions are shown in Table 2.

The means shown in the two tables are in close agreement and the significance of the figures is discussed in the foregoing statement.

NEWS and VIEWS

Electrical Engineering at Manchester : Prof. C. Adamson

DR. COLIN ADAMSON has been appointed to a newly created (second) chair in electrical engineering in the Faculty of Technology in the University of Manchester. Dr. Adamson gained a B.Sc.(Eng.) degree at the University of London in 1947, following work at the Hull and Bradford Technical Colleges. He qualified for the degree of M.Sc.(Eng.) in 1952 as a result of research work while working as assistant lecturer in the Bradford College and as research engineer at A. Reyrolle and Co., Ltd. He was appointed lecturer in electrical engineering at Manchester in 1952, and senior lecturer in 1959. He has been awarded the degree of D.Sc. this year. Dr. Adamson has been responsible over the past few years for the development in the Manchester College of Science and Technology of an extensive Power Systems Laboratory, for both undergraduate and postgraduate work, the latter forming a large sub-section of the postgraduate school in electrical engineering. His various projects have received substantial industrial support, much of it under contract. He is a representative on the British Electrical and Allied Industries Research Association, and has served on committees of the Conférence Internationale des Grands Réseaux Electriques He has recently served, by invitation, as visiting professor at the University of Roorkee, India, under the Colombo Plan, and has lectured at the Universities of Washington and Wisconsin.

Municipal Engineering at Manchester : Prof. J. R. D. Francis

MR. JOHN ROBERT DARK FRANCIS has been appointed professor of municipal engineering in the Faculty of Technology in the University of Manchester, in succession to Prof. R. J. Cornish, who recently accepted a similar appointment in the University of Delhi, India. He will take up his post in January 1962. Mr. Francis, who is forty-one years of age, gained honours in engineering at the University of London in 1940, following work in the Brighton Technical College. After service as lieutenant in the Royal Naval Volunteer Reserve he was appointed to a lectureship in the Department of Civil Engineering at the Imperial College of Science and Technology in 1946. He was appointed reader in fluid mechanics at that College in 1958. Mr. Francis has been engaged in research in fluid mechanics and has been concerned in the hydraulic design and model testing of several civil engineering works, including the Owen Falls Dam (Uganda), Tema Harbour (Ghana) and the Baldershead Dam (Durham). He held a fellowship at Woods Hole Oceanographic Research Institution, Massachusetts, during 1952–53, and has recently lectured at the Nigerian College of Technology, Zaria, and the Kumasi College, Ghana.

U.S. National Bureau of Standards : Dr. Y. Beers

DR. YARDLEY BEERS has been appointed chief of the Millimeter-Wave Research Section, Radio Standards Laboratory, Boulder Laboratories–National Bureau of Standards, U.S. Department of Commerce. He joined the staff of the Bureau in June 1960 as a consultant in atomic frequency and time-interval standards. Dr. Beers was a member of the staff of the Radiation Laboratory at the Massachusetts Institute of Technology during 1942–45; a consultant to the Brookhaven National Laboratories with the Atomic Energy Commission, 1947–59, and a teacher of physics in New York University for fifteen years, eventually achieving the status of full professor. In 1956, Dr. Beers worked as a Fulbright scholar with the Commonwealth Scientific and Industrial Research Organization in Sydney, Australia. During his stay, he was also visiting professor at the University of Sydney. His special fields of research interest are nuclear physics, microwave spectroscopy, and the electronics connected with these fields.

The Charles F. Kettering Laboratory, Yellow Springs, Ohio

DR. LEO P. VERNON has been appointed director of research of the Charles F. Kettering Laboratory in Yellow Springs, Ohio. He is well known for his work on photosynthesis and has recently returned to his former position as professor of chemistry in Brigham Young University after a year's sabbatical leave, which he spent with Prof. Hugo Theorell at the Nobel Institute, Stockholm, Sweden. Mr. Justin C. Crawford has been appointed director of laboratory administration in the Laboratory. Mr. Crawford will be responsible for all non-scientific activities and business administration. A native of Chicago, he has served with the Standard Oil Co. of Indiana since 1948, where for the past five years he has been manager of engineering administration.

Tristan da Cunha

THE island of Tristan da Cunha is the subject of a special exhibition at the Geological Museum, South Kensington. The geology is described with the aid of maps and diagrams, and characteristic volcanic rocks collected by the Shackleton–Rowett expedition during the voyage of the *Quest* in 1921–22 are also shown. A series of photographs illustrates the rugged topography of the Island, which is related to its volcanic origin. A display of projected colour transparencies is a special feature of the exhibit. Charts of historical interest, lent by the Admiralty, dating back to the 1827 Admiralty Survey by Cooling, are mounted on a separate screen. Although Tristan da

XUM

Cunha is a volcanic cone with a crater lake at the summit, there has been no previous record of eruption during historical times. The island does, however, lie on the flanks of the Mid Atlantic Ridge, which is a belt of crustal weakness extending from Jan Mayen Island, through Iceland and the Azores, southwards towards the Antarctic. The nature and extent of this submarine feature were investigated during the recent International Geophysical Year.

Award by the Fleming Memorial Fund

THE Trustees of the Fleming Memorial Fund for Medical Research have made their first grant. This is to the University of Oxford and amounts to £48,000 for the Sir William Dunn School of Pathology at Oxford, headed by Sir Howard Florey. The grant is in connexion with electron-microscopy work at the School, where the purification of penicillin was achieved and its clinical use made possible. Recently, too, cephalosporin *C*, a penicillin-like antibiotic active against some important penicillin-resistant bacteria, was discovered at the Sir William Dunn School of Pathology.

The Fleming Memorial Fund for Medical Research was launched in March this year in memory of Sir Alexander Fleming, discoverer of penicillin, to assist basic medical research. Its British and international appeal, with a target of £1 million, lasts until March 1962. So far more than £370,000 has been raised.

New Thinking in School Chemistry

THE Office for Scientific and Technical Personnel of the Organization for European Economic Co-operation arranged a seminar held at Greystones, Co. Wicklow, during March 1960, to deal with the status and development of school chemistry teaching (see *Nature*, **186**, 208; 1960), and the report has now been issued with the title "New Thinking in School Chemistry" (Pp. iii + 215. Paris: Organization for European Economic Co-operation—Office for Scientific and Technical Personnel, 1961). The contributions and discussions show that there is considerable difference of opinion on some topics. Some favour a dogmatic theoretical treatment beginning with the atom and electron as familiar modern concepts and proceeding to the development of theories of structure and bonding, the teaching being largely centred on the periodic table. What one speaker calls 'stick-in-the-mud experiments' will not take a prominent place. It is felt that distinction of *s* and *p* orbitals might be too difficult. There is sharp disagreement among those favouring this approach as to the meaning of the theories, and teachers using this method will obviously need annual refresher courses. An American speaker states that in some schools there the teaching begins with organic chemistry. Other speakers, including two from the United States, explain schemes in which models to illustrate atomic linking and molecular structure are made by the pupils. Some think that this will take too much time and will be costly. Opinions are divided on the value of films. Another group of speakers favours an experimental approach and thinks that practical work is desirable. Qualitative and quantitative analysis can illustrate general chemistry and inculcate habits of neatness and accuracy. Some suggestions for new experiments, including the use of paper chromatography in qualitative analysis, are made. Summaries of the discussions, three joint reports, and recommendations for future action close the proceedings.

Radiation Botany

THE first number of *Radiation Botany*, a new international journal devoted to plant radiobiology and related subjects, was published in September. It is edited by A. H. Sparrow together with an editorial board drawn from seven countries (*Radiation Botany*, Vol. 1, No. 1; September 1961. Pp. 100. Annual subscription rates: (*A*) for libraries, government establishments, research laboratories, etc., 140*s*., 20 dollars; (*B*) for individuals who place their order directly with the publisher and certify that the journal is for their personal use, 70*s*., 10 dollars. London and New York: Pergamon Press, 1961). This first issue of a hundred pages consists of eleven scientific contributions the subject-matter of which includes, factors affecting responses of plants to radiation, root absorption of fission products derived from underground nuclear explosion, morphological responses to gamma-rays and the influence of radiation on chiasma frequency. These and other topics dealt with illustrate the main aim of the journal, which is to be concerned with the effects of ionizing radiations on plants. It will, however, also be devoted to the more technical aspects of plant irradiation. Each contribution is usefully prefaced by abstracts in English, French and German, and papers are acceptable in any of these languages.

The journal is attractively printed in a style in keeping with the modern nature of the subject. The choice of type, paper and general layout makes *Radiation Botany* a very readable journal. Table layout is particularly good, and the line illustrations and plates are of a high standard. Though all workers in the field of radiobiology will welcome this new outlet for their writings, one may hope that genetical and physiological journals will continue to receive contributions from those who have used radiations to elucidate problems primarily concerned with those sciences.

Lanehead Field Centre

THE increasing scientific interest being shown in the 'marginal land' of the British Isles is reflected in the recent foundation of a new research centre in the Northern Pennines. The Department of Geography of the University of Durham, under the guidance of Prof. W. B. Fisher, has recently acquired a village school at Lanehead, near Cowshill, in Upper Weardale, and has speedily converted the building into an active centre for field research. Situated near the watershed of the Rivers Tyne, Wear and Tees, the new centre dominates a virgin area for field and ecological investigations. Research projects have already been started on the geomorphology, microclimate, soils and vegetation of the region, and progress is being made in the analysis of the social life of the inhabitants of the Upper Pennine Dales. In addition to giving field courses to the undergraduates of the University of Durham, the research workers (mainly postgraduate students) hope to add, in some way, to our knowledge of the social and environmental background for future development in highland Britain. Many aspects of the background still perplex the technologist and the economist; it is hoped that Lanehead Field Centre will provide at least some of the answers.

Rutgers Center of Alcohol Studies

THE Center of Alcohol Studies, which was instituted at Yale University in 1921, is being transferred to Rutgers, The State University, and will be known as

the Rutgers Center of Alcohol Studies. The transfer of the Center, internationally known for the study of alcohol in its social, biological, psychological and sociological aspects, is being made possible by a grant from the National Institute of Mental Health. It is expected that the transfer of the Center, which at Yale has been identified with the Laboratory of Applied Biodynamics, will be completed by the spring of 1962. At Rutgers the new Center will be a unit within the Graduate School of the University, the dean of which is Dr. Marion A. Johnson. The principal functions of the Center will include: (1) Research into various problems involving the use of alcohol, physiological, psychological, sociological and economic. (2) Teaching of groups with a special interest in problems of alcohol such as clergymen, public health officers, personnel workers, police, social workers, and nurses. This will be done in a summer programme, the Yale counterpart of which has been nationally famous for years. (3) Documentation of all forms of study dealing with alcohol. (4) Publication of literature dealing with the subject, including the *Quarterly Journal of Studies on Alcohol*.

Courses in Nuclear Energy

THE European Nuclear Energy Agency of the Organization for Economic Co-operation and Development (recently the Organization for European Economic Co-operation) has published a new edition of its *Catalogue of Courses on Nuclear Energy in O.E.E.C. Countries, 1961–1962* (Pp. 167. Paris: Organization for Economic Co-operation and Development, European Nuclear Energy Agency, 1961). The *Catalogue* is designed to help science and engineering students who wish to acquire a better knowledge of nuclear subjects and to find out what training facilities are available. University authorities and others wishing to compare the programmes of technical establishments in different countries will also find much relevant information. The new *Catalogue* covers the 1961–62 academic year and gives details of training courses in subjects relating to nuclear energy in some 180 Western European universities, technical high-schools and research centres. It contains a list of establishments where courses are given on subjects relating to nuclear energy; syllabuses of the main courses on nuclear physics, chemistry and engineering, with details as to lectures, length of courses, terms of admission and diplomas obtainable; and information concerning the principal items of nuclear physics and engineering equipment available for training in each establishment. Copies of the *Catalogue* can be obtained on application to the Organization for Economic Co-operation and Development, European Nuclear Energy Agency, 28 Boulevard Suchet, Paris 16.

Museum of Comparative Zoology, Harvard College

THE one hundred and first annual report of the Museum of Comparative Zoology at Harvard College for 1959–60 records the celebration of the centenary of the founding of the Museum by Louis Agassiz. Sir Julian Huxley gave a public lecture, and this was followed by a reception and a dinner. The Museum is an integral part of a major university and still maintains research and teaching, which were the main objectives of Agassiz's life. Although no major expeditions were sponsored during the year 1959–60, many trips were arranged by members of the staff for field and museum studies and for the collection of materials. A long list of publications by the staff is an indication of the great amount of research undertaken.

Cyrillic Transliteration

Scientific Information Notes for August–September (3, No. 4; 1961) reports that progress toward agreement on the use of a compromise system for transliterating Russian (Cyrillic) characters into the Latin alphabet was achieved at a meeting of representatives of scientific and engineering societies, editors, publishers, librarians and Government agencies on July 14, 1961, in Washington, D.C. The meeting was convened by the American Association for the Advancement of Science at the request of the National Science Foundation to discuss the adoption of a single, uniform transliteration system for Russian to English. The compromise system, tentatively designated as the "AAAS Transliteration System", combines features of the various systems now in use so that the transliteration of Cyrillic to Latin alphabets may be standardized. An important consideration of the proposed transliteration system was that it could be typewritten with the minimum use of diacritical marks. Systems considered in effecting the compromise were the systems of the Board of Geographic Names, the British Standards Institution, the Library of Congress–American Library Association and the International Standardization Organization. The importance and volume of Soviet scientific and technical literature have resulted in extensive efforts to translate Russian material into English. The multiplicity of transliteration systems in use has resulted in increased burdens for members of scientific and technical communities, editors, publishers, librarians and Government agency personnel. Much confusion existing in this field could be alleviated or avoided by a reduction of the number of varying transliteration systems now in use. The tentative system has been forwarded to the *Z*-39 Subcommittee on Transliteration of the American Standards Association. It is expected that an *ad hoc Z*-39 subcommittee will be formed to deal with the problem of Russian transliterations.

Learning Theory and the Therapy of Neurosis

MONOGRAPH Supplement No. 13 of the *British Journal of Psychology* (Cambridge University Press, 1961. Pp. 29. 7*s*. 6*d*. net) is entitled "Learning Theory and the Therapy of Neurosis". The author of this clear and admirably concise monograph, Mr. Ralph Metzner, provides a useful review of the literature on avoidance training and experimental neurosis in animals, and examines its relevance to some aspects of human psycho-pathology. He is particularly (perhaps excessively) concerned to analyse some recent methods of psychological treatment (so-called 'conditioning therapy') and to indicate their kinship with the animal training procedures. Although a comparative approach to behaviour and its disorders is to be welcomed, it might seem important to bear in mind the differences as well as the resemblances between animals and men. This the author signally fails to do. As a result, his work will appear to many as a gross over-simplification of the problem of neurosis and as singularly lacking in awareness of clinical reality. None the less, it has value as an unusually fair and thoughtful account of experimental work in its field and as a timely reminder that psychotherapy must eventually achieve a rational basis.

XUM

The Textile Institute

THE following awards have been made by the Textile Institute: *The Institute Medal*, to Mr. K. W. L. Kenchington, senior development officer, Directorate of Stores and Clothing Development, War Department, Royal Aircraft Establishment, Farnborough, for his contributions to the Institute in the field of clothing science and technology; *Service Medal*, to Mr. P. H. S. Bell, head of the Department of Textile Industries, Huddersfield College of Technology, and to Mr. J. Boyd, of Courtaulds, Ltd.; *Companionship*, to Mr. C. E. Harrison, president of the Federation of British Industries, and to Mr. F. M. Stevenson, chairman and managing director of Stevensons (Dyers), Ltd., and Associated Companies.

Fulbright Travel Grants

UNDER the provisions of the Fulbright programme, travel grants are available to citizens of the United Kingdom and dependent territories to go to the United States of America for academic or educational purposes, provided that they have adequate financial support in dollars for the period of their visit, and have been accepted by an American institution of higher learning for a minimum of nine months. The grants cover the cost of direct travel between a candidate's home and the American institution or university. They are available during June 1–August 15, 1962, for which applications must be received before March 16, 1962; and August 16, 1962–April 1, 1963, for which applications must be received before June 1, 1962. Application forms and further information can be obtained from the United States Educational Commission in the United Kingdom, 71 South Audley Street, London, W.1.

University News :

Birmingham

THE following appointments have been announced. *Lectureships :* R. G. Bird (civil engineering); Dr. J. S. Hough (senior lecturer in charge of the Sub-Department of Malting and Brewing in the Department of Biochemistry); A. W. Kwiatkowski (mechanical engineering); G. Martelli (physics); Dr. L. Riddiford (senior lecturer in the Department of Physics); Dr. K. R. Rushton (civil engineering); Dr. R. B. Shearn (chemical engineering); N. B. W. Thompson (physical metallurgy). *Research Fellowships :* T. Duthie (Wilmot Breeden Fellow in the Department of Mechanical Engineering); Dr. H. Kaliszer (mechanical engineering); B. K. Gazey (electrical engineering); R. J. Heitzmann (chemistry); Dr. A. E. Pedler and Dr. C. A. Barson (senior Research Fellows in the Department of Chemistry); Dr. B. Henderson (physical metallurgy); G. R. Orgam (industrial metallurgy); B. Walker (industrial metallurgy); Dr. S. Swierszczewski (nuclear physics); J. R. Turcan (engineering production); C. J. H. Williams (James Watt Research Fellow in the Department of Mechanical Engineering).

Leeds

Mr. G. L. Bibby and Dr. A. E. D. Heylen have been appointed to lectureships in the Department of Electrical Engineering, and Dr. M. Holmes to a lectureship in the Department of Civil Engineering.

Liverpool

THE City of Liverpool College of Technology has been admitted to affiliation with the University of Liverpool for a period of three years from October 1, for the purpose of teaching pharmaceutical chemistry for courses leading to the ordinary degree of B.Sc. and to the degree of B.Sc. with honours in pharmacology. During the period of this affiliation the head and four members of the Department of Pharmacy of the College staff will be recognized as teachers of the University.

London

THE title of professor of social statistics in the University of London has been conferred on Mr. C. A. Moser in respect of his post at the London School of Economics and Political Science.

Manchester

PROF. P. J. COLLARD, of the University College of Ibadan, has been appointed professor of bacteriology and director of the Department of Bacteriology in succession to Prof. H. B. Maitland. Prof. Collard will take up his duties in April 1962.

College of Aeronautics, Cranfield

DR. R. L. APPS, of Atomic Power Construction, Ltd., has been appointed to the post of lecturer in the Department of Materials; Mr. A. G. Simms, lecturer at Leicester College of Technology, has been appointed to the post of lecturer in the Department of Production and Industrial Administration; Mr. J. W. Mair has been appointed Research Fellow in the Department of Materials; Dr. A. Younger, of Richard Thomas and Baldwins, Ltd., has been appointed Research Fellow in the Department of Materials.

Announcements

PROF. J. C. MITCHESON, professor of mining at the Royal School of Mines, Imperial College of Science and Technology, and a member of the Geological Survey Board since 1959, has been appointed chairman of the Board in succession to Sir Walter Drummond.

MR. B. N. HUGHES, at present editor of *Technology* and special writer to *The Times* on technical and technological education, has been appointed a director of Messrs. Chapman and Hall, to succeed the late Mr. Geoffrey Parr as chief editor for scientific and technical books.

A SYMPOSIUM on "Low Temperature Carbonization of Non-caking Coals and Briquetting of Coal Fines" is being organized by the Regional Research Laboratory, Hyderabad, India, during November 20–22. Further information can be obtained from the Director, Regional Research Laboratory, Uppal Road, Hyderabad 9, A.P., India.

THE Anatomical Society of Great Britain and Ireland will hold its annual general meeting at St. Mary's Hospital Medical College, London, during November 23–24. The meeting will be devoted to papers on "Cytology of Nervous Tissue". Further information can be obtained from the secretary, Prof. R. J. Harrison, Anatomy Department, London Hospital Medical College, Turner Street, London, E.1.

THE British Insecticide and Fungicide Conference will be held at Brighton during November 7–10, under the auspices of the Association of British Manufacturers of Agricultural Chemicals. Further information can be obtained from Mr. W. A. Williams, secretary, Association of British Manufacturers of Agricultural Chemicals, Cecil Chambers, 86 Strand, London, W.C.2.

NEW SOURCES OF ENERGY

THE United Nations Conference on "New Sources of Energy" was convened for the exchange of experiences and new ideas on recent progress in the applications of solar energy, wind power and geothermal energy, and held in Rome during August 21–31. The term 'new' is not perhaps the best description of these sources of energy which have been used by humanity for many centuries, and is used to emphasize that their development has been largely ignored or regarded as uneconomical in the past but that they should now be included in any survey of power resources. These three forms of energy are very different in their characteristics; a geothermal energy source is a gift for any nation fortunate enough to possess such a site, for although there are many problems involved in harnessing this form of energy, its utilization follows well-established procedures. Solar and wind energies are ubiquitous, though varying in intensity in different localities and at different times; they are both 'low intensity' sources having a maximum energy flux of the order of 1 kW./m.^{2}. The latter, however, is in the form of mechanical energy for which Nature has paid the toll of the Carnot cycle efficiency, while the former is in the form of heat, and mankind must pay the toll to convert this to mechanical or electrical energy. Consequently, although the conversion of solar radiation to electrical power on a small scale would be advantageous, the major form of utilization should be as heat at low or high temperatures as required.

It is not possible to make detailed comment here on the technical proceedings at a conference attended by some 650 delegates from more than fifty countries and at which some 250 papers were presented, about half being on applications of solar energy. All the papers were summarized by *rapporteurs* in sections; these reports were discussed later and a final summary was given at the concluding session for each form of energy under consideration. Two visits were arranged during the conference. One was to a demonstration of a solar power plant, 2–5 kW. capacity, designed by the National Physical Laboratory of Israel. This uses air-inflated plastic mirrors and covers in the form of cylinders about 12 m. long which are aligned east and west, and only need moving occasionally to allow for the seasonal change in the position of the Sun. Steam is produced at a temperature of 150° C. and the heat transferred in a storage vessel to vaporize an organic working fluid which is expanded in an impulse turbine, condensed and returned to the heat exchanger. This design has been reduced to the simplest essentials and has an estimated cost of 1,000 dollars per kW. installed, and a power cost of 5 cents per kW. hr. based on 6 per cent interest rate and 15 years life. The other visit, of contrasting nature, was to the geothermal station of Larderello. This power system, in use before the Second World War, has now been extended to a capacity of 2×10^9 kW. hr. annually, using steam at an average pressure of 5 atmospheres absolute and a temperature of 200° C. The first impression is the unusual sight of gigantic cooling towers rising from surrounding countryside, with a relatively low power-house and a maze of snake-like pipes conveying steam from the distributed bore-holes. Use of geothermal steam is complicated by the high gas and mineral content of the steam, so that most of the power stations operate by heat transfer to a pure water circuit from which the steam is passed to the turbines. This simplifies the gas removal problem and provides for effective recovery of the minerals, mainly boron compounds. Conference papers were presented on the recent developments in the use of geothermal power in New Zealand and Iceland, as well as prospective applications in Japan, South America and other volcanic zones.

Wind-power units of small output are well established in agricultural regions. The problems involved in larger units of 100–500 kW. which could generate electricity for a grid supply are still under investigation, but the tendency would appear to be towards a direct-drive propeller unit geared up to the generator by mechanical or hydraulic transmission and with automatic voltage control.

The papers on solar energy applications covered many fields, such as water and house heating, crop drying, distillation, power production by thermal cycles, refrigeration, air conditioning and cooking. The direct conversion of radiation to electricity by thermocouples, photo-voltaic cells and thermionic devices was also discussed, and recent developments in solar furnaces. Details of these processes were described more fully in my report on the World Symposium on Applied Solar Energy, Arizona, 1955 (*Nature*, **177**, 110; 1956). Since that date there has been development in new designs and improved equipment, especially as regards economical construction, but no really new principles have emerged. The number of large-scale installations for water heating and distillation at present existing remains very small, and there is still insufficient detailed information available to select the most satisfactory designs for various purposes. Most of the designs in the fields mentioned above have been developed independently, and are still in the experimental stage. Large-scale commercial developments seem limited to water heaters in Israel, and some large-area solar-stills in the United States. One of the proposals given prominence at the conference was that a series of experimental stations should be established in various countries where the scaling up from the experimental models to the larger industrial installations could be developed and in this connexion mention should be made of the Brace Experimental Station at Barbados, West Indies, recently established by McGill University. The extensive funds available for research on space travel and artificial satellite powering will make possible the development of many schemes for the direct production of power from solar radiation which can be adapted for terrestrial use.

Perhaps it will be more profitable in the space available to discuss the economic and social implications of solar energy utilization. Much of the discussion at the conference concerned the under-developed nations, which might be described more politely as non-industrial, and how these could be helped by the industrial nations. One possible procedure suggested would be for the industrial countries to manufacture and sell equipment to the non-industrial countries.

XUM

but individuals could not afford to purchase such equipment without a national subsidy in the first instance. The preferred alternative would be for the industrial countries to develop very simple designs of equipment which could be made in the country where required and thereby establish village industries. The first stage might well be along communal lines in villages, for baths, refrigeration and electricity generation, rather than attempting to accomplish these for individual dwellings. The importance of simplicity was emphasized, since in solar appliances the capital cost per unit of energy collected is more significant than the thermal efficiency of conversion. The sociological feature is the difficulty of introducing a new method of doing something already accomplished by other means, for example, to replace refuse-burning cookers by solar cookers, but where the application is completely new, as for electricity generation or refrigeration, there is not the resistance of tradition.

As regards industrial countries, solar energy can only be integrated with other energy sources, and water heating is the most feasible application. The prospects of success are greatest in the Mediterranean countries, decreasing but not necessarily being non-existent in the more temperate countries. Much consideration was given to the assessment of potential resources in all the fields of energy considered. These data are particularly lacking as regards solar radiation and a much closer network of recording stations is required to assess the availability on a micro-climatic basis.

The final conclusions would appear to be:

Geothermal power is analogous to hydro-power, and obviously should be developed in the few localities where such sources exist, provided that the power can be transmitted to centres of population. Wind-power is an established factor in agricultural areas, but development is needed before large wind-power generators can be constructed. Utilization of solar energy is the most difficult of all to establish. Protagonists must have faith in the possibilities, some of which are not yet proved. Similar situations have, however, arisen in other technologies now fully established and justified, for example, space travel, where the initial difficulties have eventually been overcome as unforeseen developments occurred. The urgent needs are international co-operation to establish satisfactory designs of equipment and the training in Europe and the United States of personnel from the user nations who can return to promote investigations in their own countries.

The general impression is that the development in six years is not as great as would have been expected. It is, however, encouraging to find that there are many people of high academic ability in various countries who have enough faith to devote their whole, or at least a considerable proportion, of their time to the attainment of a deeper knowledge of the underlying principles of all these sources of energy and in particular of solar energy devices, in order to raise the existing standard of living in under-developed countries. Co-ordinated planning during the next six years should promote a significant increase in applications.

Long-term predictions for the future are necessarily uncertain, but there is no doubt that energy demands will grow more rapidly than the world population, which is expected to double by the year 2000, and that all sources of energy should be actively investigated and integrated. The conference and discussions arising will undoubtedly promote these objectives, and lead to more successful international co-operation in future developments.

H. Heywood

MICROWAVE MEASUREMENT TECHNIQUES

A CONFERENCE on the general subject of microwave measurements was held at the Institution of Electrical Engineers during September 6–8. A large audience of scientists and engineers from many countries assembled to consider some fifty papers covering all aspects of modern microwave measurement.

In his introductory lecture, Prof. H. E. M. Barlow, of University College, London, stated that microwave measurement techniques could be grouped broadly under the following headings: measurements using field probes; measurements depending on resonance arrangements; attenuation and phase shift measurements; noise measurements and power measurements; and that the general limits of accuracy of microwave measurements at the present day were of the order of ± 0·1 per cent for impedance measurements, ± 1 per cent for power measurements, and about ± 0·01 db. up to 3 db. for attentuation measurements.

Subsequent sessions of the conference considered most of these groups in detail.

Prof. Barlow also described two new devices utilizing the Hall effect for microwave power measurement. In the first, a thin indium arsenide crystal is rotated continuously in a wave-guide. The resulting Hall E.M.F. in the crystal is thus modulated at the rotation frequency, unlike the parasitic thermoelectric and rectified E.M.F.s occurring at the crystal contacts, from which it can therefore be separated by a filter. The second device was demonstrated, and comprised an array of Hall crystals in cascade which could be used to measure high-power densities in free space. The multiplicity of crystals helps to cancel out the unbalanced rectifier effects occurring at the contacts.

A supporting paper by R. E. Larson of the Boulder Laboratories of the U.S. National Bureau of Standards outlined the calibration facilities offered by the Electronic Calibration Center of the Bureau. These are ultimately intended to cover the measurement of power, impedance, frequency, attenuation and noise over the full frequency-range 300–40,000 Mc./s.

An interesting paper by Prof. C. Süsskind and P. O. Vogelhut, under the title "Cavity-perturbation Measurements of the Effect of Microwave Radiation on Proteins", described experiments concerned with the biological effects of microwave radiation on living animals. Irradiating power densities of the order of 0·1 W./cm.^{2} produce significant changes of body temperature in mice, and accordingly the 'tolerance' level for human beings is accepted as 0·01 W./cm.^{2}. Apart from the heating effect, microwave irradiation causes other biological

changes—in particular, it brings about a change in the hydration-rate of proteins. The measurement problem involved is one of determining the values of complex permittivities of the order of $60 + 30\,j$, within times of the order of milliseconds. These are found by measurements of the Q and resonant-frequency shift of a resonator through which the sample is passed at a constant rate.

J. E. Pearson and J. A. Jane of the Radio Research Station (Department of Scientific and Industrial Research) underlined the need for power density monitors to measure power densities of the order of 1-20 mW./cm.^{2} for health hazard purposes. Calibration of such meters requires a source of microwave energy of known power density and the authors described such a source utilizing parallel plate transmission line for frequencies between 0·3 and 1·5 Gc./s.

Further papers presented on the first day dealt mainly with the measurement of impedance, admittance and reflexion coefficients, although papers dealing with noise measurement and problems of measurement associated with travelling wave tubes were also included. The former group of papers was largely concerned with the less-conventional types of measuring equipment: reflectometers; rotary standing wave indicators and 3-probe lines, together with an equipment for instantaneous impedance measurement described by P. Hargrave and S. J. Robinson of Mullard Research Laboratories, which utilizes seven coaxial hybrid rings, capable of giving the polar components of an impedance directly to within ± 10 per cent.

On the second day the subject of power measurement was reviewed in an introductory paper by Prof. A. L. Cullen of the University of Sheffield. The present position could be broadly summarized by stating that most instruments for the measurement of microwave power are accurate to within about ± 2 per cent, although considerable difficulties still exist in providing reliable apparatus for the measurement of small powers, particularly at the higher frequencies. Among hitherto unutilized effects suggested by Prof. Cullen which might find application in future power-measuring devices were the use of electrostrictive and magnetostrictive materials to convert electromagnetic field energy into mechanical movement and the use of the saturation-level of maser-type devices, which occurs with increased pump-power, as a reference standard. A further similar scheme, using second-order saturation effects in ferrites, was put forward as being possible by Dr. P. J. B. Clarricoats of the Queen's University of Belfast. Prof. Cullen also suggested that it should be possible to make a wattmeter depending on the detection of the change of electromagnetic angular momentum occurring when circularly polarized waves are incident at a conical surface.

"A Survey of Microwave Power Measurement Techniques employed at the National Bureau of Standards (U.S.A.)" was presented by G. F. Engen, who dealt in detail with problems of bolometric measurement errors and mount efficiency.

J. A. Lane gave a "Comparison of Power Measuring Standards at Microwave Frequencies", indicating that measurements of the efficiency of numerous bolometer mounts conducted in various countries, whether determined by microcalorimeters, film bolometers, impedance measurement or torque vane wattmeters, were all in good agreement at power-levels of the order of 1–2 mW. The latter three methods exhibited errors generally of the order of ± 1–2 per cent, while rather better accuracy was possible with the microcalorimeter.

An interesting new instrument for the measurement of power was described by Dr. E. Acs of Budapest. The power to be measured is absorbed in a film bolometer element terminating a waveguide and the consequent heating of the surrounding air causes air convection currents in an adjacent channel. The convected air stream impinges on a vane, the resulting deflexion of which is dependent on the power dissipated in the film.

Further papers by Prof. Barlow and D. Woods of the Ministry of Aviation described respectively a suggested method for the measurement of high-frequency power using a thin crystal of indium antimonide placed edge-on to the incident wave (it can be shown that in the absence of reflexions from the crystal edges the Hall voltage is directly proportional to the transmitted power), and a coaxial millivoltmeter/milliwattmeter which uses a silicon diode to measure voltage and power to within 0·5 per cent and 1·5 per cent, respectively, at all frequencies from d.c. to 1 Gc./s.

L. Lewin and G. H. B. Thompson of Standard Telecommunication Laboratories, Ltd., introducing the afternoon session, read a paper emphasizing the difficulty of "Measurements in Microstrip". The reasons for the present unsatisfactory state of affairs are first, the lack of a simple theory on the basis of which components can be designed and, secondly, no satisfactory insertion type detector yet exists.

Two papers presented by R. J. Turner of the Post Office Research Station (one in collaboration with D. L. Lisney) covered equipment used for the measurement of group delay and insertion loss of wave-guide networks in the 3·8–4·2 Mc./s. band for use in the testing of components for microwave links used for multi-channel telephony and television transmission. Other papers dealt with the measurement of wave-guide attentuation, measurement of dispersion of disk-loaded wave-guide and of propagation coefficients in wave-guides with unknown but symmetrical input and output coupling. Among a group of papers devoted to attenuators, a paper on "Resistive Attenuators and their Calibration", by D. A. J. Walliker of Decca Radar, Ltd., dealt specifically with the use of metallized glass vanes and their mounts. Such attenuators with micrometer adjustment can yield accuracies of 0·04 db./micrometer division at 40-db. level in size 10 guide, with resetting accuracies up to an order of magnitude better than this. An attenuator of this type in size 16 guide has retained its calibration within ± 0·03 db. over a five-year period of laboratory use. Practical calibration systems for S and X band attenuators and their general specifications were also described.

The final morning was devoted specifically to millimetre wave techniques, the papers presented falling into two distinct groups, namely, those concerned with H_{01} circular wave-guide transmission and those concerned with other topics. The former group included a paper by R. Hamer and R. J. Westcott of the Post Office Radio Research Laboratory on the measurement of attenuation in short lengths of circular guide. The Q factor ($\sim 10^6$) of a length of guide under test, when used as a resonator, was measured by comparing the resonator response curve when excited by a swept frequency oscillator with that of a standard reference resonator of lower Q. The response curves of the two resonators are matched by adjustment of the display sweep frequency and

amplitude, the attenuation constant being deduced from the readings of the attenuators used to achieve this control. The ratio of measured to theoretical attenuation for plain copper guide and copper or aluminium helix guide was of the order of 1·4 at 35 Gc./s. for a 2-in. diameter guide in all cases.

R. F. Skedd of the Standard Telephone Laboratories discussed the generation, and measurement of attenuation, of unwanted modes associated with long-haul wave-guides, while a paper by Dr. A. E. Karbowiak, G. Craven and V. H. Knight of the same establishment described an equipment for the automatic recording of wave-guide attenuation. This apparatus was capable of recording the variation of attentuation with frequency over the whole of the 40–80 Gc./s. band in a space of about ½ hr.—an operation which, without the degree of automation used, would require some thousands of independent sets of measurements. An additional paper by Dr. Karbowiak and Mr. Skedd was concerned with an instrument for the accurate determination of relative surface losses in the millimetric band.

Among the other papers, Dr. J. Brown and Dr. A. P. Anderson of University College, London, dealt with the use of a single crystal as a harmonic generator and as a detector at a wave-length of 4 mm. Such an arrangement with the addition of a simple ferrite modulator has been used to measure the impedance of wave-guide components at 4 mm., as well as to determine the permittivity of liquid dielectrics.

W. L. Birch of the General Electric Co. Hirst Research Centre described a milliwattmeter capable of detecting changes of power ~ 10 μW. for the 70–80 Gc./s. band in which the power to be measured is absorbed in a carbon load, the temperature rise in which is detected by a thermopile with which it is in thermal contact. A further wattmeter for use at millimetric wave-lengths was demonstrated by Dr. H. A. French of A.S.W.E., Portsdown, and Prof. A. L. Cullen. The instrument was of the oscillating torque vane type, was capable of measuring power as low as 100 μW., and, like its predecessors, was also capable of absolute calibration.

The construction of Fabry–Pérot interferometers and their use for the measurement of permittivity of such substances as TiO_2 was then considered in two papers by Dr. W. Culshaw and M. V. Anderson of General Telephone and Electronics Laboratories Inc., California, and by Dr. J. S. Seeley and J. C. Williams of Queen Mary College (University of London).

The final session was concerned with measurements on materials. Prof. Barlow described a method for the measurement of permittivity of high-loss dielectrics in which the specimen under test was located in an evanescent guide coupled to a resonant cavity, thus avoiding the disturbance of the field which normally results when the specimen is located within the cavity. For dielectrics with small losses Prof. G. B. Walker and S. P. Luthra of the University of British Columbia used 'ghost modes' for the determination of complex permittivity. A disk specimen of the dielectric is located in a wave-guide and a resonant mode of the specimen—the 'ghost' mode—is excited, the latter being a mode in which the fields outside the specimen are strongly evanescent and thus contain little stored energy. The limitations of the technique were discussed and a further paper by Prof. Walker and Messrs. J. M. Free and M. E. B. Moffat of the University of Oxford showed how these as well as normal cavity techniques could be used for the investigation of the properties of titanium dioxide and magnesium titanate ceramics at *S*, *X*, *J* and *Q* bands. Dr. Hamer and Mr. Westcott described a method of measuring the complex permittivity of dielectric films while R. B. Nichols and A. E. Brown of Elliott Bros. spoke about an apparatus for grading dielectric cubes according to their exact permittivity. The cubes, used for the fabrication of Luneberg lenses, possess permittivities lying between 1 and 2 which are determined by noting the position at which resonance occurs, as a movable plunger sweeps the cube through a test cavity. Further papers by Dr. Clarricoats and by C. P. Aron of Morganite Research and Development, Ltd., described a simple wave-guide method for the determination of the permittivity of cylindrical dielectric rods and an apparatus for the measurement of the tensor susceptibility of ferrite materials.

G. D. Sims

PROGRESS IN MICROCHEMICAL METHODS AND TECHNIQUES

THE 1961 International Symposium on Microchemical Techniques, organized by the Metropolitan Microchemical Society of the United States under the sponsorship of the Commission on Microchemical Techniques, Section of Analytical Chemistry, International Union of Pure and Applied Chemistry, was held during August 13–18, at Pennsylvania State University, University Park, Pennsylvania. The excellent accommodation and facilities made possible an informal atmosphere of fellowship and individual contacts. The purpose of the symposium was to provide a forum for the interchange of information and ideas among technologists from all parts of the world concerning new methods and techniques or unique applications of microchemical or microanalytical interest.

The symposium was inter-disciplinary both with respect to the interests of the almost five hundred technologists attending and the technical programme of ninety-five papers and lectures. The symposium was truly international in character with more than twenty countries represented. Travel of many speakers to the United States was supported by a generous research grant from the National Institutes of Health, U.S. Public Health Service.

The technical sessions and plenary lectures were supplemented by a three-day commercial exhibition of equipment, apparatus, specialities and literature, by six panel discussions, film sessions on the history of microchemistry and research advances, a practical demonstration session, a popular lecture, and by various social events.

The plenary lectures were of salient interest. H. Lieb (Austria) delineated the history of microchemical techniques and A. A. Benedetti-Pichler (United States) the development of microchemistry in the United States. D. Van Slyke (United States) surveyed the evolution of micro-gasometric methods

of analysis, a field in which he has long pioneered. E. Sawicki (United States) considered the present status of micro methods for the sensitive and selective detection and determination of aldehydes and aldehyde-yielding substances, emphasizing his own successful studies. R. Belcher (England) examined methods developed for the determination of elements and functional groups in organic compounds using sample weights of 30–50 μgm., an area in which he and his co-workers have made notable advances. N. D. Cheronis (United States) discussed the challenges encountered in the characterization of organic compounds at the microgram and especially the submicrogram range. It appears that many characterization reactions are so slow at concentrations less than about 10^{-5} molar that operationally they may be considered as not occurring. The technical papers covered the entire gamut of microchemical and microanalytical interests, and, in almost all cases, presented novel and previously unpublished research findings.

A special session on thermo-microscopic methods due to L. Kofler was prefaced by a short talk by A. Kofler (Austria), which reviewed the course of her husband's hot-stage experiments. A. Lacourt and N. Delande (Belgium) reported an extended investigation of the identification of some sixteen amino-acids by the hot-stage observation of eutectic temperatures with twenty-five different components, of which oxalic acid and dicyandiamide appear especially good. Of the general papers on microscopy, that of L. W. Gamble (United States) was especially noteworthy; it introduced a new approach for the characterization of aliphatic hydrocarbons, namely, microscopic study of their crystalline urea and thiourea adducts. E. Wiesenberger (Germany) described how the electron microscope could be used somewhat analogously to the light microscope for the detection of substances via the observation of chemical reactions. The reactions, largely precipitation of metal ions with suitable reagents or electrical or chemical reduction to metals, are conducted on the special carriers used in electron microscopy within a field of 0·004 mm.^{2}. E. Waldmann (Switzerland) considered the systematic differentiation and identification of crystalline deposits in body tissue by microscopic tests.

Inorganic microanalysis was represented by many significant papers. Those of H. Flaschka and P. O. Sawyer (United States) and of A. Ringbom (Finland) emphasized the value of photometric titrations for metal ions present in small amounts or at extremely low concentrations using either ethylenediaminetetraacetate or a chelating chromogenic agent as titrant. Sawyer and Flaschka also described an elegant micro- and ultramicro-photititrator using as the light-sensing element a commercial semiconducting photodiode responding in such a small angular range that titrations may be performed in open daylight. J. Zýka (Czechoslovakia) recommended lead tetraacetate as an oxidizing titrant for the potentiometric titration of various metals and organic compounds in acetic acid or in an aqueous medium containing mineral acid. R. Přibil and F. Vydra (Czechoslovakia) based a simple oxidimetric microtitration of cobalt on the finding that iron(III) oxidizes chromium(II) in the presence of 1,10-phenanthroline or 2,2′-bipyridyl, which also masks many interfering cations by complexation. L. Erdey and G. Svehla (Hungary) described the chronometric determination of metal ions based on their catalysis of suitable reactions in which the elapsed time is recorded between mixing and the appearance of the Landolt effect; the peroxide–iodide reaction permits the determination of 0·1 μgm. of molybdenum within 99 per cent accuracy.

Quantitative elemental organic analysis, as a major subject for microchemical research, was considered in many papers. In the combustion analysis for carbon, hydrogen and sulphur, C. Maresh and co-workers (United States) proposed a gas chromatographic finish. H. Malissa and E. Pell (Austria) reported on their recent investigations on the complete automation of the micro-determination of carbon, hydrogen and sulphur. A. M. G. Macdonald and Y. Gawargious (England) gave the results of a comparative study of various high catalytic fillings in the combustion analysis for carbon and hydrogen; cobaltic oxide appeared to be the most satisfactory for general work. J. A. Kuck and co-workers (United States) described an instrumented combustion method for carbon and hydrogen in which carbon dioxide is assessed by infra-red absorptiometry and hydrogen by thermal conductivity.

G. Ingram (England) introduced a direct ignition in an oxygen-filled chamber at 850° C. for the determination of carbon, hydrogen, halogens and sulphur; the combustion products are swept into appropriate absorbing reagents and determined. The method requires only 30 min. or less per sample and is applicable to difficult materials, such as organic fluorine or phosphorus compounds. W. G. Guldner and A. L. Beach (United States) based a rapid micro determination of oxygen on pyrolysis of the organic sample in a helium stream in an induction-heated graphite crucible and oxidation of the carbon monoxide, after trapping of unsaturated compounds, to carbon dioxide, which is absorbed in barium hydroxide solution and determined conductometrically. J. L. Brownlee, jun. (United States), presented an intriguing approach to the determination of oxygen in organic compounds involving a neutron-induced reaction in an atomic reactor, namely: $^6Li(n,\alpha)^3H$; $^{16}O(t,n)^{18}F$; fluorine-18 is separated and determined radiochemically.

Noteworthy papers in organic functional group analysis included that of J. E. Zarembo and M. M. Watts (United States) on the determination of the carbodiimide function, of E. Sawicki and co-workers (United States) on a highly sensitive spectrophotometric determination of azuelene, and of W. Merz (Germany) on the determination of active hydrogen using alkyl-substituted metal aluminium hydrides. C. A. Rush and J. I. Miller (United States) proposed barium isopropyl methylphosphonate as a rigorous reference standard for carbon, hydrogen, phosphorus, barium and alkoxyl. S. R. Palit and P. Ghosh (India) based a determination of acids, bases and salts at micromolar concentrations in benzene on the colour behaviour with rhodamine and certain other dyes previously extracted by benzene under specific conditions. M. Bobtelsky (Israel) considered his recent research work with microheterometry in work on the reactions and nature of large organic molecules, including dye lakes and metal–protein complexes.

Physical measurements on the micro scale also received attention. The use of a dynamic flow technique in place of the usual static drop method was recommended by C. L. Ogg and co-workers (United States) for the thermistorized determination of molecular weight. W. Simon and co-workers

(Switzerland) described refined thermistor circuitry and instrumentation for the routine, precise determination of molecular weight using a sample weight of only 2·5 mgm. for a molecular weight of 500. P. D. Garn and J. E. Kessler (United States) demonstrated that thermal analysis is feasible for sample weights less than 1 mgm. by the use of appropriately designed sample holders.

In the session on nucleonics, G. F. Morrison and J. F. Cosgrove (United States) described the application of neutron activation to the sensitive determination of the copper activator in single crystals of electroluminescent zinc sulphide weighing less than 500 μgm. B. A. Thompson (United States) considered how radiochemical techniques and especially neutron activation could be applied to the determination of the principal components, trace impurities and thickness of metal films a few angstroms in thickness and one square centimetre in area; such films are encountered in the performance and design of electronic devices, including vacuum tubes. M. T. Kelley (United States) reviewed the present status of the analysis of highly radioactive reactor fuels and spent products using liquid samples of 5–700 μl.; coulometry, spectrophotometry, flame photometry, emission spectroscopy, titrimetry and radiometry, and density measurements are feasible by remote controlled, automatic or 'slave' manipulated operations. A. Holasek and co-workers (Austria) explained the difficulties and operational problems encountered in the synthesis of milligram amounts of fatty acids and esters labelled with carbon-14, and considered salient factors involved in the selection of suitable synthetic methods.

Chromatographic methods were the subject of many interesting papers. E. G. Wollish (United States) considered critically the accelerating interest in alumina and silicic acid for thin-layer chromatography in the separation and identification of organic compounds, including drugs. H. K. Mangold and co-workers (United States) exemplified how thin-layer chromatography acts as a valuable analytical and preparative tool in lipid chemistry. L. S. Ettre and W. Averill (United States) demonstrated how new gas chromatographic instrumentation using capillary (Golay) columns and ultra-sensitive detectors permit the direct analysis of trace components in samples of micro-size, and set forth criteria for the selection of methods and instrumentation. L. Wiesner and W.-J. Schmidt-Küster (Germany) described the gas-chromatographic performance of a new integrating counter-detector using a flow-type proportional counter containing a source of ionizing radiation and used with a suitable scaler. T. S. Ma and M. Gutterson (United States) recommended gas chromatography in the determination of several organic functional groups. S. Patton (United States) considered the identification of food flavours using a diversity of techniques including gas, paper and column chromatography, and the study of the dinitrophenylhydrazones of volatile keto-compounds. Paper chromatography was applied by B. Bobrański and L. Syper (Poland) to the identification of drugs containing unsaturated radicals following reaction with mercuric acetate. A. K. Dey and E. J. Singh (India) explained the behaviour and application of various complexing agents in the separation of metal ions by the Weisz ring-oven technique.

The contemporary position of small-scale methods in the teaching of chemistry at the secondary school and collegiate levels was assessed in a special session with speakers from Northern Ireland (C. L. Wilson, N. N. Gilpin), England (J. B. Headridge, H. Holness), and the United States (N. D. Cheronis, T. S. Ma and J. A. Kuck). A. Steyermark (United States) considered the training of chemists in industry in quantitative organic analysis.

When the *Proceedings* of this symposium become available as a single volume (Interscience, early 1962), the interested technologist will be able to ascertain the position of microchemistry and microanalysis for 1961 in the spectrum of scientific activity, and to comprehend the research trends that will yield the 'to-morrow' of these disciplines. In brief, microchemical and microanalytical research faces continuing challenges with ever smaller samples, ever more minute components, coupled with demands for adequate accuracy, precision and rapidity. Improved instrumentation, automation and new techniques and reagents, coupled with re-assessment of present methods and approaches, will energize further advances.

A. J. Barnard, jun.
John Y. Steel

INSECT POLYMORPHISM

A SYMPOSIUM on polymorphism in insects, organized by the Royal Entomological Society of London, was held at the Imperial College of Science and Technology, London, during September 21–22. The president, Prof. G. C. Varley, was in the chair at the first meeting and expressed a hope that special attention would be directed to the agencies promoting and controlling this type of variation.

Prof. O. W. Richards, in a general introduction, stated that polymorphism is very widespread among the insects and occurs in all the major orders. The metamorphosis of insects, itself a mode of polymorphism, perhaps makes them especially prone to such variation. There are two principal types of polymorphism: the genetic type, where two or more genotypes co-exist in a state of balance, and the induced type, in which some variable environmental factor such as population-density, food, day-length or temperature leads to the development of two or more kinds of individual in a species.

Dr. E. B. Ford, in an account of the theory of genetic polymorphism, stated that the standard examples are only special cases of a more general phenomenon which is often overlooked when the characters involved are not easily visible. This is the tendency for selection to favour the heterozygote over both homozygotes when, after some change in the environment, a rare major gene begins to spread. The favourable effects of the gene will tend to become dominant and the unfavourable ones recessive, and the heterozygote will have all the advantages and none of the disadvantages of the homozygotes. Where polymorphism results from the interaction of several genes, the whole complex is controlled by a supergene, the link between the units of which may be strengthened by a chromosome inversion. The original effects

of the controlling mechanism are always liable to evolutionary change by selection of the remainder of the gene-complex.

Dr. P. M. Sheppard then reported on recent work on polymorphic mimetic swallowtail butterflies. Knowledge of the way in which mimicry arises and is maintained makes possible a number of predictions as to how the polymorphism should be controlled genetically. These predictions can then be compared with the genetics of the polymorphic mimics so far as these have been unravelled. The most striking results have been obtained in the African *Papilio dardanus*, in which several different forms of female, some mimetic and some not, correspond to a single type of male. Recently, methods have been devised for breeding the butterfly artificially in England and a genetic analysis combined with field data on population statistics show a good agreement with theoretical predictions.

Prof. T. Dobzhansky dealt with the dynamics of chromosomal polymorphism in *Drosophila*. In the giant chromosomes of the larval salivary glands one can recognize individuals which as a result of inversions of parts of chromosomes have different gene arrangements. Several chromosomal types may occur in one population and their proportion may be constant, vary seasonally, or over longer periods, or geographically. Such changes in proportion may directly demonstrate the action of natural selection and allow calculation of the selective advantages of the polymorphs. The mere order of the genes may itself be an element in the fitness of a polymorph, but perhaps more often an inversion by suppressing crossing-over preserves an arrangement which gives an advantage to heterozygotes. Several of the field phenomena can be reproduced in cage populations. The proportions of the polymorphs are easily modified by changes in the environment or in the genetic background. Crosses with stocks from widely separated sources have an unpredictable effect on the proportions of the inversions because of the large number of alternative backgrounds provided.

The next four contributions were concerned with familiar examples of non-genetic polymorphism, namely, in Hymenoptera, in termites, in aphids and in locusts. Prof. C. D. Michener dealt with the first topic and showed that in only a few bees and in ants is the worker caste sharply different from the queen. In most social bees and in wasps the differences are slight and overlapping, occurring mainly in behaviour and physiology, but in several ants the worker caste is itself polymorphic. In the least differentiated castes of some halictine bees, the slight differences arise in the adult stage but the castes of all other Hymenoptera, except perhaps *Melipona*, seem to depend on differences in nutrition, sometimes interacting with temperature. At some point in development, however, the caste becomes determined. In *Pheidole*, the workers produced from a queen's first batch of eggs are small, even if they were well fed; the type of worker is thus determined in the egg, but perhaps here through the nutrition of the queen which laid them. The selective advantages of the castes of advanced insect societies is clear but their evolution is anomalous, since the first step seems to be the reduction of fertility in some individuals. However, it appears that economy of labour and better defence of the nest make even the most primitive social organization (one queen and one worker in some halictine bees) more viable than isolated queens.

Dr. M. Lüscher gave an account of social control of polymorphism in termites, one of the most astounding of all forms of social regulation in animals. Termites may have up to seven different castes in most of which each sex is equally represented. The newly hatched insect is capable of developing into any of the castes by a series of moults. Some forms may also have regressive moults leading back to an earlier stage in the series. Caste determination mainly acts by directing moults in the required direction, but the workers may also reduce a caste to its correct proportions by selective cannibalism. The direction of moults is influenced by active substances or pheromones which are exchanged among members of the colony. This is most clearly established in the inhibition of the reproductive caste by members of the same caste already present. Lüscher has shown by fixing a queen in a screen so that her head is in one colony and her abdomen in another, and then varnishing over parts of her body, that the active substance comes from the anus. Complete inhibition, however, requires the presence of a male as well as a female reproductive, so that there seem to be two substances. Another one is probably involved in the inhibition of the soldier caste. There are also stimulating substances; more males, for example, being produced in a colony with a queen but no king than in one without either. Termites are only responsive to such influences for part of the time intervening between each moult. The substances probably act through an effect on the ductless glands of the insect. There may be as many as eight different pheromones, but none is as yet chemically determined.

Dr. A. D. Lees then described the clonal polymorphism of aphids, especially in *Megoura viciae*. Aphids may reproduce viviparously and parthenogenetically or be fertilized and lay eggs. They may be winged or apterous and there is more than one kind of wingless female. The full development of some of the morphal characters may appear gradually during several successive generations. Other morphs (for example, sexual-parthenogenetic) are strict alternatives. Controlling mechanisms are almost all environmental, acting through the endocrine system. Aphid reproduction is extremely precocious so that an embryo will already have ovulated at its deposition and it is thus necessary to determine which of the three generations is being affected. Males can be suppressed by suitable temperatures or in some species photoperiod but are otherwise produced in a characteristic percentage. Egg-laying females are produced at photoperiods of less than 15 hr. and temperatures of about 15° C. Winged forms arise as a result of crowding and this is not an indirect effect acting through the host-plant. There is also an 'interval-timer' which does not allow sexual forms to be produced for some time (50–60 days at 15° C.). By selecting the first and last members of each generation to start separate lines, it can be shown that the interval is a matter of days and not of generations.

Continuous polymorphism in locusts described by Dr. J. S. Kennedy is different from most of the other examples in that the morphological differences are relatively slight and intermediates are often commoner than the extremes. Yet not only the morphology but also almost every aspect of behaviour and physiology are somehow affected. Phase transformation is largely controlled by population-density, which may act directly or on the previous generation. Genetic

make-up, temperature and other factors may also have some importance. The solitary phase may be regarded as more juvenile than the gregarious one, and recent experiments have shown that the prothoracic gland which normally determines a juvenile morphology persists throughout life in the *solitaria* although disappearing early in the adult *gregaria*. Dr. Kennedy suggested that *solitaria* is adapted to a 'soft' habitat where food is available without much movement. *Gregaria* is suited to a 'hard' habitat which can be exploited only by the continuous movement which is typical of the phase. The polymorphism as a whole allows the species to exploit a much wider habitat than one phase could alone.

Dr. J. H. Sang, speaking on the environmental control of mutant expression, dealt with a developmental problem. In the course of growth a gene may produce a graded response in an enzyme which nevertheless has a discontinuous effect on adult development. Thus the gene 'antennaless' in *Drosophila* determines 0, 1 and 2 antennæ but nearly always perfect structures. This is because the action of the gene occurs during a very short but well-defined period, namely, between the seventieth and eightieth hours of larval growth. This is shown by transferring larvæ of various ages to a different culture medium, for the effect of the gene can largely be nullified by suitable food. Such examples suggest how polymorphism might be determined by either a genetic or an environmental switch mechanism, in which influences at the right state of growth may produce large and discontinuous changes in the adult.

Prof. V. B. Wigglesworth, in a tentative synthesis, drew analogies between the polymorphism of cells in different parts of the body, the polymorphic aspect of insect metamorphosis and the phenomena which the other speakers had discussed. He showed that there seemed to be a complete transition from variation in which genetic determination was almost absolute to that in which external environmental factors decided between several alternatives.

The animated discussion which followed the invited contributions did not bring forward many really new points but was chiefly concerned with the relative importance of genetic and non-genetic factors in particular examples. Terminology was also discussed, and some speakers were unwilling to speak of polymorphism unless the variations were clearly discontinuous. The suggestion, however, was made that the best distinction was between situations in which there is selection for increased variability as opposed to the more normal case where deviations are discouraged.

O. W. Richards

MOLECULAR BIOLOGY AND POPULATION STRUCTURE

A SYMPOSIUM on "Molecular Biology and Population Structure" was held at Norwich on the afternoon of September 4 at a joint session of Sections D (Zoology) and K (Botany) of the British Association for the Advancement of Science. The aim of the symposium was not so much to present new discoveries as to demonstrate to the audience the interrelations of recent discoveries in these fields, and to discuss some of their implications.

The symposium was opened by Dr. T. Alderson (British Empire Cancer Campaign), who described what we know of the structure of deoxyribonucleic acid (DNA) and brought out some of the evidence that genetic differences as we observe them are the resultant effects of differences of DNA molecules. Dr. J. A. Pateman (University of Cambridge) followed with a discussion of the evidence that gene differences have their effects by influencing the structure of protein molecules, though in discussing, at the end of his review, the recently discovered class of genes that appear to have the capacity of "turning on and off" whole metabolic sequences, he pointed out that there is evidence that these do not operate by producing proteins. These new regulator genes may well prove of major importance for our understanding of differentiation. Prof. H. Grüneberg (University College, London) then made the connexion between enzymes and more conventional characters by describing some beautiful examples, from his work with mice, which showed how the little invisible differences in chemical structure may be magnified during embryological development into the larger more obvious differences with which the study of genetics began.

At this point the symposium turned to what at first sight was another topic altogether, when Prof. J. M. Thoday (University of Cambridge) described how a variety of genes may be maintained in a population if that population were exposed to a variety of demands. Dr. P. M. Sheppard (University of Liverpool) then went on to discuss some of the evidence, illustrated from his work on butterflies, that the variety of genetic types in a population are not only directly adapted to the variety of conditions which the members of the population can exploit to promote survival, but must also be adapted to one another. The population is an integrated unit, and over the generations the genetic contents of different components of the population are judged not only by their individual success but also by the degree to which they co-operate with one another when brought together in sexual reproduction. The chromosomes of a population have not only to have genetic properties that are to be understood in terms of the needs of individuals but also other genetic properties only to be understood in terms of the population as a whole. Among other things the chromosomes of different individuals have to be co-adapted to one another if the population is to function successfully over the generations.

Dr. C. A. Clarke (Royal Infirmary, Liverpool) followed Dr. Sheppard with a little of the evidence that all these principles apply to man just as much as to *Drosophila*, or butterflies.

Prof. J. M. Thoday gave the final paper. He pointed out that the two most striking discoveries of modern genetics had been brought together in this symposium.

The first discovery concerns the molecular nature of the genetic material. It has, of course, long been supposed that genes are molecules of some sort or another, but the precise knowledge of their molecular nature we are now acquiring is one of the most spectacular achievements of biology.

XUM

The second discovery is of a different kind and was less expected. Both mathematical studies concerning the way genes might be supposed to behave in populations, and the biochemical concept that genetic variation means variation of the enzyme content of individuals, led us to expect that a population should be rather uniform genetically, each individual having much the same genetic make-up as other individuals. Further, this is the expectation we seem instinctively to tend towards in our attitude to our own populations, in our concept that the people we know and with whom we have a sense of belonging are essentially like ourselves.

It was this belief in the relative genetic uniformity of populations that gave such interest to the pioneer investigation of cases of obviously polymorphic populations which formed evident and striking exceptions to this expectation. Polymorphic populations are populations in which two alternative genes are both frequent, whereas simple theory would lead us to expect one to oust the other so that the population would tend to uniformity. One case of polymorphism, sickle-cell anæmia in man, mentioned in a number of the papers, provides an example where population genetics and molecular biology tie up closely together.

Study of such polymorphisms not only led to their explanation but also helped us to make the discovery that cases of polymorphism such as Dr. Sheppard discussed are far from being exceptional. They are only the striking and obvious examples of what is a normal phenomenon. For the expectation of genetic uniformity is not in the least borne out by fact in any well-studied population. The facts are quite contrary. Populations of sexually reproduced species consist of a wide variety of genetically different individuals and the genetic differences are in fact usually greater than the differences that we can see. So great is this genetic variety that we may go so far as to say that no two individuals (apart from identical twins) are genetically alike. This applies not only to flies and butterflies but also to man; in fact the evidence that it is true for man is probably more complete than it is for any other organism. Each of us is genetically different from all the others. This genetic or biochemical individuality is one of the major facts of life.

Along with the discovery of this genetic, or biochemical, individuality has come explanation of it at least in general terms. The basic explanations are two.

The first invokes the fact that we live, and all our ancestors have lived, in an environment that changes slowly with time. It changes as the climate changes, and it changes as other organisms change. We, that is the species to which we belong, have therefore inherited from the ancestral populations from which we have descended the means to change as the environment changes, a means that permits change in directions the necessity of which could not be foreseen. Sexual reproduction. which permits the generation of a variety of genetically different individuals, and death, which permits the elimination of those individuals less-well adapted or no longer adapted to the environment, provide that means of change. Sexual reproduction, however, only permits the generation of a variety of genetically new individuals, it does not ensure it unless there already is a variety of individuals. Hence, in the long term, natural selection in a changing environment must result in the continued survival only of populations that contain and always contained enough genetic variety to be able to produce the new variants that new environments require.

The second explanation is one which has developed more recently. It is not an alternative to but supplements the first. This explanation invokes the fact that the environment in which a population lives is not uniform in space. Different parts of the population meet different environments. Hence we must expect that, in different parts of a population, genetically different kinds will tend to be the fitter, so that variation of the environment in space must lead to the selection of genetical variety within the population. The results of the laboratory work done to test this view lead to the conclusion that this is a most powerful factor; selection for variety is astonishingly successful.

We therefore begin to have an understanding of the function in life of this surprising biochemical variety that has been found in natural populations. It is the basis of the means of maintaining the population successfully in conditions that vary with space and change with time, and it is the prerequisite of innovation.

Prof. Thoday went on to discuss at some length some of the implications of this knowledge as it applied to man, pointing out that, though we know little of the genetics of normal variation in man and need much research on it, we do know enough to be sure that within the range of what are called normal humans there is every sort of variety to be found. Each of us is different in molecular constituents that affect all classes of characteristics, whether physical or mental. How much vitamin we need to remain healthy, what our normal blood pressure is, what our taste preferences may be, how sociable we are, how quick in the uptake, how liable to fall to temptation; most things will be affected by our innate molecular constitution as well as by our upbringing.

This is not to deny the plastic adaptability of *Homo sapiens*. Plasticity of the individual is one of the striking properties of man, and environment in the general sense, which includes other people and upbringing in particular, can have great effects on the characteristics of human individuals. Nevertheless, we may be sure that the effect that a particular environment and upbringing will have on one man will differ from its effects on other men. Their genetic differences will ensure that this is so.

Now this is a consequence of our knowledge to which we shall find it difficult to adjust ourselves, but it has a bearing on almost every important human topic we discuss, because most important problems in some way or other involve the question of the justice of inequality in human society. Contemporarily, questions of equality in educational opportunity and the justice of giving the more promising. in so far as we can assess them, better opportunities in grammar schools, provide an excellent example of a topic in which the facts of genetic variety are seldom dispassionately considered. But we must, some time, face the question of the relation between such topics and our knowledge that most, if not all, differences between people, and between classes of people, are in part genetic as well as being in the remaining part environmental in causation. Our sense of justice goes against accepting the fact that some people are biologically better endowed than others from the point of view of their capacity to profit from education and their capacity to return to society a profit on the cost of their education. But it is a fact nevertheless:

life is not just in this sense. It is time that we realized that all men are of equal value just because all men are different. Some are better for some purposes, some for others and their variety, which is the basis of the richness of life, is at their initiation there, a cardinal fact of life, arising from the uniqueness of the molecules that form their basic genetic endowment. Society should be organized to profit from that individuality, not suppress it, however inconvenient it may be to bureaucrats and mass-production marketers. Further, if we could but accustom ourselves to the fact of human variety we might less often fall into the errors to which the type concept leads in our attitude to the differences between classes and between races. The hidden genetic variety within a group is much more striking than are the evident differences that distinguish groups within a species. J. M. Thoday

BIOLOGY IN BRITAIN

Progress within any major branch of science is inevitably uneven. Thus, there has been in the post-War period very rapid scientific advance in certain aspects of biological science particularly amenable to study by modern chemistry and physics. Unaware of the outstanding problems of biological science this has led some authors to advance such judgments as "modern science has all but wiped out the borderline between life and non-life"[1]. Such over-enthusiastic assessments in their extreme form amount, as Commoner[2] has pointed out, almost to a declaration that biology as a separate science is no longer needed. Perhaps more dangerous is the reaction which now calls for a unifying of biological science by amalgamating existing disciplines in a way which, in practice, effects their contraction or elimination in favour of new, fashionable but frequently very restricted 'subjects'. Further, the call for such changes is often prefaced by the contention that established biology teachers are out of touch with modern biology. Dr. Wyatt's recent article[3] is tinged with this approach.

His statement "the classical biology of pre-War years has changed little" is placed in a context which clearly implies its decreasing relevance to the main stream of biological thought rather than emphasizing that its slower development arises both from the resistance of many of its major problems to rapid solution and a lack of research resources. His contention that the American biology graduate is better equipped to teach other subjects (a rather doubtful role), to read the journals and to do and to direct research is too sweeping, despite the excellence and breadth of the biological education offered in some American universities. His contention that British students may learn more about modern biology from *The New Scientist*, the Penguin Science Survey and television than they gain from the isolated subject studied at a university at best displays lack of knowledge of many existing degree courses and of the separate and complementary role of important media of general scientific education.

Dr. Wyatt's solutions for the difficult problems facing many of us who are concerned with training biologists are too easy. For example, he finds the course for the diploma of technology in applied biology far, I imagine, beyond the hopes or intentions of its originators, to be more suited for academic biology and research than are the courses (and this presumably means any and all courses) offered at the universities.

It should be emphasized that there is ample evidence that, in Britain and other countries, teachers of biology are at present deeply involved in a re-assessment of their teaching programmes. For example, in March of this year, at a meeting attended by almost all the professors of botany in British universities, very careful consideration was given to such problems as: the sixth-form courses most appropriate for those intending to become biologists (emphasizing the need for basic training in the physical sciences and in mathematics); the rapidly changing character of university courses and the need for improved facilities, particularly for teaching in biochemistry, physiology and genetics; the influence of present Government statements on the recruitment to biology of first-class students and the financing of fundamental biological research. Further, new science buildings are being used to bring under one roof, and hence into close contact, groups of related departments, as exemplified by the new Biology Building at Birmingham and the Natural Sciences Building in my own University College. Biologists who, in their undergraduate courses and postgraduate work, have specialized in biochemistry, biophysics, microbiology and genetics are increasingly being appointed to existing departments of botany and zoology.

Dr. Wyatt rightly raises the question of the establishment of departments of biology as against continuing along a pathway of increasing fragmentation. This is in line with the recent memorandum of a Royal Society Committee consisting of fifteen of its Fellows, representative of all the major fields of biology. Insulated, introspective departments, if entrusted with the teaching of any branch of biology, will inevitably tend to deny to their students the value of the collaborative activity which has so evidently been effective in making many recent advances in our science. Nevertheless, there are dangers and difficulties in fully and quickly effecting the proposed re-organization. One of the difficulties hampering effective biology teaching is only now being eased by the present rapid expansion of higher education. Only recently in many institutions has it become possible to build up towards a teaching staff in biology whose training and research interests are sufficiently varied to give the desired range of related courses. One of the dangers is revealed in Dr. Wyatt's approach when, specifically mentioning as staff members, an ecologist, a physiologist, a biochemist, a microbial geneticist, a plant pathologist and perhaps an embryologist, he contends that courses in zoology, botany, biochemistry and microbiology, as well as in chemistry, can be made available by a biology department. The danger here, in its extreme form, is that 'molecular biology', instead of being visualized as a major and active field of discovery, is put forward as already synonymous with the whole of biology. This danger is, in fact, at present a matter of deep concern to many distinguished American biologists. The Royal Society Committee, taking a broader view

of the scope of biology, clearly recognized the need for some 6–8 professorial appointments together with a substantial number of non-professorial staff in order to establish a really effective unit for teaching and research in the biological sciences.

H. E. Street

Department of Botany,
University College of Swansea.

[1] Asimov, I., *The Intelligent Man's Guide to Modern Science*, **2**, *The Biological Sciences* (Basic Books, New York, 1960).
[2] Commoner, B., *Science*, **133**, 1745 (1961).
[3] Wyatt, H. V., *Nature*, **191**, 960 (1961).

If biologists wish to avoid "molecular biology", "in its extreme form", then we must devise courses that will attract potential physical scientists. Can we be complacent when biochemists are becoming more divorced from biology and when more of the better students study neither botany nor zoology? While I argued for experimental biology, at the same time I emphasized the greater need for studying live animals and plants: not only in field studies but also in the laboratory. It is not that our established biology lecturers are out of touch, but that our organization, syllabuses and examinations are. Because I believe that biology is relevant, I wish to see more physical scientists study it.

The memoranda of the Royal Society and of the professors of botany are both in private circulation; other biologists would be glad to read them.

H. V. Wyatt

Department of Bacteriology,
University of Leeds.

CONQUEST OF HUNGER

A SYMPOSIUM on food supply and population was an important feature at the meeting of the British Association for the Advancement of Science in 1960 in Cardiff. Papers bearing on this theme were delivered by a number of leading authorities, followed by discussions (see *Nature*, **188**, 9; 1960). The papers have now been published by the British Association in a booklet called *Hunger: Can it be Averted?* edited by Sir John Russell and Dr. Norman C. Wright*. The booklet, with a preface by Sir John, contains the following articles: "The Current Food Supply Situation and Current Trends", by Dr. Norman C. Wright; "Population Prospects and their Implications", by Prof. D. V. Glass; "Economic Conditions for Greater Output", by Prof. W. A. Lewis; "Land Use and Food Production", by Prof. L. Dudley Stamp; "Industrial and Technological Needs", by Lord Fleck; "What Limits Food Production?", by Prof. H. D. Kay; "Some Conclusions", by Prof. P. M. S. Blackett. There is an appendix, with seventeen figures, entitled "Vast Population Increases Intensify the Battle Against Hunger".

The booklet presents the main facts about food supply and population lucidly and without emotional bias, as might be expected from the high status of the contributors. What are these facts? If world population continues to increase at the present rate it will double itself by A.D. 2000, and nothing likely to happen within the next few decades will reduce this rate of growth, due largely to 'death control', which will become steadily more effective. In fact, the rate of increase will probably accelerate as the expectation of life rises in the developing countries and the saving of child life—the field in which modern medicine and public health have achieved their most striking successes—produces a population structure particularly favourable to high fertility.

At present large numbers of people in the world are under-fed and badly fed, so that existing food supplies are inadequate. To feed the much larger population of 1980–2000, much more food than is now being produced will be needed. The quantities required, at given levels of consumption, can be roughly estimated. Potentially, they can be made available by distributing surpluses, bringing new land under cultivation and increasing yields. For economic and political reasons the contribution of surpluses is unlikely to be important. There are wide areas, at present unproductive, which can be brought under cultivation at great effort and cost, but the most promising possibilities lie in increasing yields from land already under cultivation. Sufficient technical knowledge already exists to increase yields enormously in most parts of the world, and this knowledge is being extended year by year. There is plenty of dramatic evidence showing what can be achieved, for example, by using more fertilizers. It is a familiar fact that yields of rice in Japan are 3–4 times greater than in India, a difference due to the large amounts of inorganic fertilizers used in Japan, combined with improved seed varieties, controlled irrigation and efficient cultural practices. The crux of the matter is whether solutions can be found to the economic, political, social, educational and administrative problems which prevent technical knowledge from being effectively applied. As Prof. Blackett puts it: "to raise the low level of much of the indigenous agriculture and to bring under cultivation more of the abundant cultivable land requires a massive educational and social advance combined with heavy capital investment in communications, housing, schools, agricultural implements, fertilizers, irrigation and drainage and, as Prof. Kay has pointed out, barbed wire". The key lies in the "massive educational and social advance".

Departments of agriculture in many countries are at present under-staffed and often ineffective, and great efforts are needed to establish extension services to bring modern techniques to cultivators. Food crops for local consumption usually receive less attention from agricultural departments than cash crops. Technical assistance to developing countries in fields concerned with food production should be given on a much greater scale. These and numerous other facts with an important bearing on the situation are discussed by the contributors.

While the booklet deals adequately with population trends and prospects, it does not go deeply into the basic issue of population control. It is primarily concerned with practical and inescapable problems of food supply and population which mankind must face in the near future. On these it says nearly all that can be said and much that has been said before. The picture is indeed clear enough. In the preface,

* *Hunger: Can It Be Averted?* Edited by Sir E. John Russell and Dr. Norman C. Wright. Pp. v+80. (London: British Association for the Advancement of Science, 1961.) 5*s*.

XUM

Sir John Russell comments: "the broad conclusion was that hunger need not be feared for at least the next forty years and this result could be achieved on present knowledge if only it were properly applied. . . . These optimistic conclusions from such an authoritative group come as a great relief after the pessimistic gloom that has long surrounded the subject". The booklet may not produce this impression on all its readers, who should be many.

W. R. Aykroyd

PLANT PHENOLICS GROUP OF NORTH AMERICA

THE recent increase of interest in the biology and chemistry of the phenolic constituents of plants becomes immediately apparent on inspection of the lists of contents of many of the world's biological and chemical publications. However, workers in the various fields embraced by an interest in the plant phenolics are widely scattered throughout departments of botany, biology, biochemistry, chemistry, microbiology, pharmacy, pharmacology and forestry, in universities, research institutes, government agencies and in industry. There is consequently almost no common meeting place for people with such interests, with the result that there is little discussion and exchange of information between workers of the different disciplines.

In 1956, the Plant Phenolics Group was founded in Great Britain as an informal organization with the aims of promoting "the advancement of the knowledge of phenolic and related constituents of plants in respect of their chemistry, function, biosynthesis, effect on plant and animal physiology and pathology, and the application of such knowledge in agriculture and industry". Since its founding the Plant Phenolics Group has been very successful in achieving these aims, holding two or three meetings a year. The proceedings of many of these meetings have been published.

At the ninth International Botanical Congress in Montreal in 1959, discussions with various biochemists and physiologists interested in plant phenolic substances led to the idea that a sister organization be formed in North America. The largest problem with forming such a group on this continent, of course, results from its geographical size, which would prohibit such a group from meeting more frequently than once a year.

An organizing committee, consisting of Dr. T. A. Geissman (University of California), Dr. V. C. Runeckles (Imperial Tobacco Co. of Canada) and Dr. G. H. N. Towers (McGill University), distributed a letter to interested persons in 1960, the response to which was so enthusiastic as to encourage the committee to make plans for an inaugural meeting. This was held at Colorado State University, Fort Collins, during August 31–September 1, and took the form of a symposium on the biochemistry of plant phenolic substances. The meeting was sponsored by the National Science Foundation under the direction of Mr. G. Johnson of Colorado State University, and Dr. Geissman. The papers presented were: "Recent Studies on the Structures and Bitterness of the Flavonoid Glycosides of Citrus" (Dr. R. M. Horowitz, U.S. Department of Agriculture, Albany, California), "Biosynthesis of Plant Phenols" (Dr. S. A. Brown, National Research Council, Saskatoon), "Naturally Occurring Biflavonyls" (Dr. N. Kawano, University of Nagasaki, Japan), "Metabolic Fate of Phenolic Substances in Animals" (Dr. A. N. Booth, U.S. Department of Agriculture, Albany), "Role of Plant Phenolics in Disease Resistance and Immunity" (Dr. I. Uritani, Nagoya University, Japan), "Melanin and its Formation" (Dr. G. A. Swan, University of Durham) and "Estrogenic-like Substances in Plants" (Dr. E. M. Bickoff, U.S. Department of Agriculture, Albany). It is hoped that the proceedings of the symposium will be published.

More than sixty delegates attended the symposium, the last session of which was devoted to founding the Plant Phenolics Group of North America. The officers elected were: *president*, Dr. S. H. Wender (University of Oklahoma); *vice-president*, Dr. L. Jurd (U.S. Department of Agriculture, Albany); *hon. secretary-treasurer*, Dr. V. C. Runeckles.

It was agreed at the first meeting that membership of the Plant Phenolics Group of North America be open to all persons interested in the Group's aims, on application to the secretary, Dr. V. C. Runeckles, P.O. Box 6500, Montreal, Quebec.

V. C. Runeckles

RESEARCH WITH TROPICAL PLANTS

THE Tropical Products Institute of the Department of Scientific and Industrial Research is continuing its work on the examination of plants from tropical territories, and a recent report outlines the scope of investigations carried out during 1960*.

Any new sources of drugs could be of great economic benefit to the countries to which the plants are indigenous, and the Institute's activities follows representations from overseas governments for technical assistance in this field. Already 314 plants have been tested, including specimens from North Borneo as well as samples from Kew Gardens. A number of alkaloid-containing plants have been found and are being subjected to further tests. The Director of the Institute reports the expansion of economic and long-term research work by the Institute to meet the needs of rapidly developing tropical countries. This reflects a trend away from short-term inquiries in order to help newly established laboratories overseas with their research and development problems. Even more important is the demand for qualified manpower in the territories themselves, and the Institute has helped by sending visiting scientists to 39 overseas territories and by training research workers from five different tropical countries. More than 900

* Department of Scientific and Industrial Research. *The Report of the Tropical Products Institute Committee, with the Report of the Tropical Products Institute.* Pp. viii + 40. (London: H.M.S.O., 1961.) 3s. net.

inquiries for advice and assistance were received from 62 countries during the year. While only 10 per cent of these inquiries required laboratory investigation, they took up about three-quarters of the Institute's advisory effort.

At the request of certain Malayan pineapple producers, the Institute is investigating the reason for differences between the flavour of Malayan pineapple juice and Hawaiian and South African juices. This may be due either to the effect of local canning process on the flavouring constituents or to varietal differences inherent in the plants. Many countries have a seasonal surplus of fruits and vegetables, and the advice of the Tropical Products Institute has been sought on methods of making better use of these supplies. Although small-scale canning of surplus fresh fruits and vegetables is not generally recommended as the quality and quantity are unlikely to be sufficient for effective processing, advice was given to Fiji and Nyasaland on how to set up a small-scale canning line; to Kenya on packing pineapple slices in plastic cases; to Singapore on making cashew apple wine; and to Aden and Tanganyika on producing tomato paste or purée.

In many parts of the tropics, surplus coconuts are made into copra by drying the kernel in the open air or in special kilns. Most copra is made by the small peasant farmer and quality control is difficult. As moisture content is a critical factor in the keeping qualities of copra, the Institute is examining the effectiveness of a simple moisture meter, which was originally developed at the Pest Infestation Laboratory for testing grain. A simple portable meter of this kind could easily be used by local farmers.

For some years the United Kingdom has been looking to the Commonwealth for new sources of naval stores (turpentine and rosin). Three hundred gallons of pine oleo-resin from Mauritius were examined at the Institute to see whether it would yield good commercial quality turpentine and rosin. The samples compared favourably with American products, and it is hoped that Mauritius will be able to improve its economy by starting a small naval stores industry based on existing plantations.

Work has also begun on an improved type of solar still and the first model is being tested in Malta. In addition, water-heating equipment using solar energy, developed at the Institute in London, is now being tested under field conditions in Malta. It comprises two fully instrumented flat-plate water heaters for research purposes as well as domestic flat-plate heaters for the roofs of private houses and blocks of flats.

FORMATION OF AIR–WATER FROTHS

By J. RENNIE and W. SMITH

Warren Spring Laboratory, Department of Scientific and Industrial Research, Stevenage, Hertfordshire

FOR the absorption of gases in liquids or for distillation on a large scale, effective contact between the two phases is obtained by dispersing gas or vapour at a high rate into a pool of liquid. Froths or foams are formed, giving a good rate of heat or mass transfer across the phase boundary. The generation of these froths is in no way dependent on surface activity: froths can be formed from pure components (Fig. 1), so that the surface tension is uniform in the pool. These froths are not stable: they collapse the instant the gas supply is removed.

One very common and simple device used for the dispersion of gas is the sieve plate. This is a flat plate, drilled with evenly spaced holes, from 1/16 to 3/8 in. in diameter, occupying 5–20 per cent of the total area of the plate. Liquid is generally run across the plate, being maintained in depths of 2–8 in. by weirs, and the gas is dispersed upwards through the holes.

In studying the performance of sieve plates, we have concentrated on froths formed by dispersing air in water. The complicating effects of mass or heat transfer between the phases, and the resulting non-uniform distribution of physical properties in the pool, have thereby been avoided. Preliminary work has been mainly concerned with devising techniques for measuring accurately the surface area and density of the dispersions[1]. It has been found that two kinds of dispersion are obtained, depending on gas-rate; relatively low gas-rates produce a stable, immobile foam of large bubbles, and high gas-rates produce an actively moving froth. It is the froth which is of practical interest, owing to the higher distillation or absorption-rates obtained.

Examination of single short-exposure photographs of froth produced over a range of conditions has shown that the important physical characteristics of the froths, such as density or surface area per unit volume, remain the same whether the plate used has one or many orifices; in particular, the size of bubbles or cells in the mass of the froth is almost uniform, and is independent of the diameter of the orifice.

As may be seen in Fig. 1, before coalescence near the surface of the pool, the bubbles in the froth are all about the same size, and smaller than the bubbles formed at the orifices. Leibson *et al.*[2], who investi-

Fig. 1. Froth formed over a multiple-orifice sieve-plate in a shallow pool. Air in water

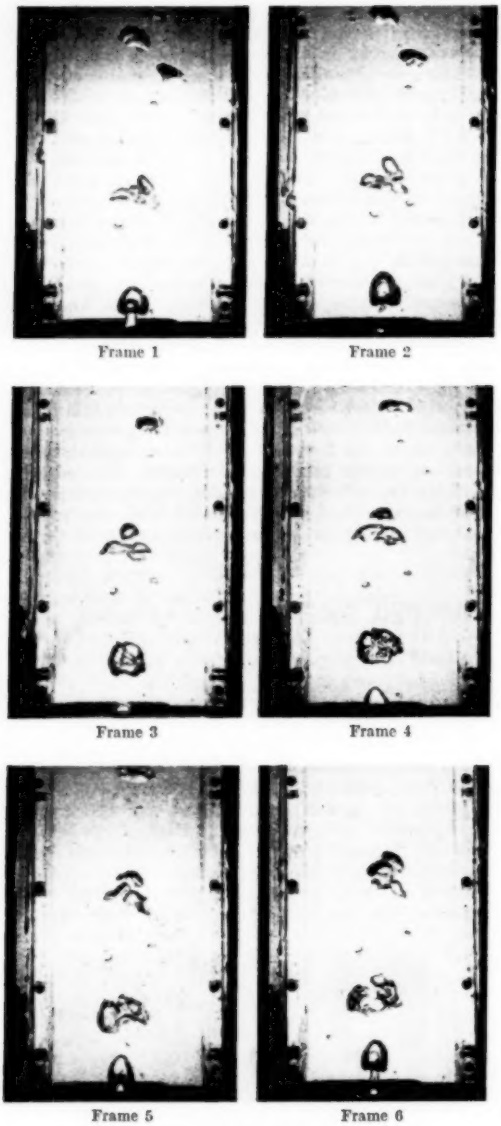


Fig. 2. Progressive break-up of bubbles. Frames at intervals of 1/64 sec.

gated bubble formation at a single orifice, concluded that the small bubbles are produced by sudden shattering of the large bubbles initially formed, and this is indeed what appears to happen when the formation of the froth is followed by a slow-speed ciné camera or by eye. Helsby and Tuson[3], in a study of bubble behaviour at low gas-rates, observed the onset of coalescence between pairs of bubbles near a single orifice. To examine the process in more detail, however, we have taken ciné-photographs of the break-up of bubbles formed by single- and multiple-orifice plates at 64 frames/sec. with synchronized lighting, supplemented by high-speed photographs at 3,000 frames/sec.

Fig. 2 illustrates the behaviour of bubbles before and during disintegration; the sequence of six photographs was taken at intervals of 1/64 sec. These bubbles are formed at a single orifice of 1/8 in. diameter, in a deep pool of liquid. In frame 1 a bubble has been formed and became detached from the orifice. A second bubble is shown penetrating the first bubble, forming a neck of gas connecting it to the orifice. When the 'neck' is detached from the orifice, it may remain attached to the main bubble but separated by a film of liquid, or it may be an integral part of the main bubble. Sometimes no distinct neck is formed, but a second, smaller bubble follows the first in quick succession. In nearly all cases the neck or the second bubble overtakes the main bubble and, being usually of smaller diameter, penetrates it. Frames 2, 3 and 4 show the increasing agitation of the combined bubble as it begins to rise through the liquid. In some photographs filaments and films of liquid from the penetrating bubble can be seen within the penetrated bubble. Frames 5 and 6 show the cells of the combined bubble beginning to move apart and form separate bubbles. The further progress of the break-up can be seen by following the upper group of bubbles. On the rare occasions when a bubble is released without being penetrated by a neck or a second bubble, it remains whole until the bulk of the froth at a higher level is reached.

With slight variations (for example, at very high gas-rates more than two bubbles may interpenetrate) this mechanism of disintegration operates under all conditions which give frothing in the air–water system; the eventual size of bubble or cell of froth is almost constant. The effect of changing the interfacial tension by using other gases and liquids, and the condition where the interfacial tension depends on the life of the bubble, as when mass transfer is taking place, are now being examined.

[1] Calderbank, P. H., Evans, F., and Rennie, J., *Proc. Intern. Symp. Distillation* (Inst. Chem. Eng., London, 1960).
[2] Leibson, I., Holcomb, E. G., Cacoso, A. G., and Jacmic, J. J., *J. Amer. Inst. Chem. Eng.*, **2**, 296 (1956).
[3] Helsby, F. W., and Tuson, K. R., *Research*, **8**, 270 (1955).

EFFECT OF COPPER ON PRODUCTS OF ORCINOL OXIDATION

By Dr. T. F. SLATER
Department of Chemical Pathology, University College Hospital Medical School, W.C.1

THE addition of alkali (final normality approx. 0·01) to a solution of orcinol containing small amounts of copper (approx. 10^{-6} *M*) leads to the rapid production of a red-brown colour. Colours so formed in sodium hydroxide or in ammonium hydroxide are very similar providing the reaction time is short (about 1 hr.). If the alkaline orcinol reaction is allowed to proceed for a much longer time (say, several days) a marked difference between the spectra in sodium hydroxide and ammonium hydroxide becomes apparent due to the formation of the dye orcein in ammoniacal orcinol. Orcein is a

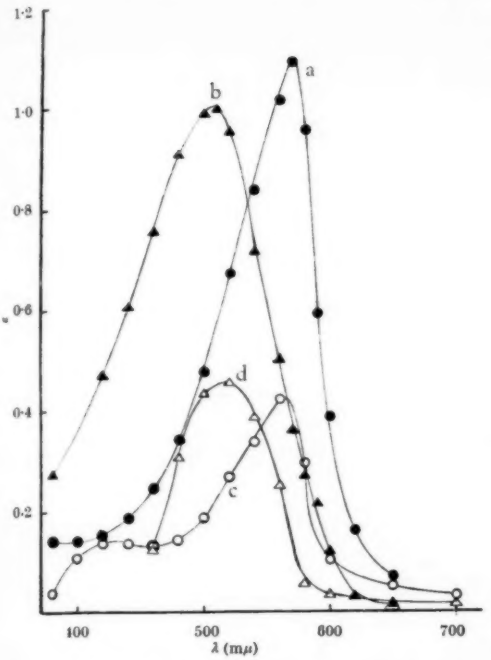


Fig. 1. Absorption spectra obtained using a commercial sample of orcein *a*, in 0·1 *N* alkali ; *b*, in 0·1 *N* acid ; *c*, and *d*, are the alkaline and acid difference spectra for the oxidation of orcinol in the presence and absence of copper ions calculated as follows : two flasks containing ammoniacal orcinol were incubated for 4 days at room temperature ; one flask, *A*, contained in addition 1 ml. 1 per cent sodium diethyldithiocarbamate and the other flask, *B*, contained 2 μgm. copper ions. After 4 days the absorption spectra of both solutions were determined and the alkaline difference spectra calculated by subtracting spectrum *B* from *A*. Both solutions were then acidified to approx. 1 *N* with HCl and the spectra again measured. The acid difference spectrum was calculated by subtracting spectrum *B* from *A*

mixture of related products which can be separated by paper chromatography[1]. A commercial sample of orcein obtained from British Drug Houses, Ltd., gave absorption maxima in acid and alkali at 510 and 570 mμ respectively (Fig. 1).

An unexpected feature of these results was that copper ions inhibited the formation of orcein ; this is illustrated in Fig. 1. It can be seen that the acid and alkaline peaks formed in the virtual absence of copper ions are identical with the results found with a commercial sample of orcein. It would thus appear that the complex reactions leading to the formation of the orcein dyes are inhibited by traces of copper present in the reaction mixture.

The reaction between orcinol, alkali and small amount of copper with short times of incubation can be used as a quantitative method for determining low concentrations of copper. Lambert[2] has published an assay method for copper based on the catalysis of resorcinol oxidation, but confined himself to assays in pure solutions and did not investigate the applicability of the method to biological samples where trace metal analysis is often required. The applicability of the copper catalysis of phenol oxidation as a method for copper analysis in biological samples has therefore been investigated Since this work was started before seeing the report of Lambert[2], it is concerned solely with the oxidation of orcinol. Some of the early work on this investigation was done using borate and phosphate buffers but these were later replaced by the more satisfactory pyrophosphate buffer suggested by Lambert[2].

The assay system used was to incubate 2 ml. 1 per cent orcinol, 5 ml. pyrophosphate buffer (0·1 *M*, *p*H 12), copper solution (0–1·0 μgm.) and water to give a final incubation volume of 25 ml. at 30° C. for 10–30 min. with shaking at 120 shakes/min. Lambert proposed stopping the reaction by acidification, but this greatly decreases the extinction value and a more convenient procedure was found to be the addition of a strong copper chelator (versene) at the end of the incubation. The addition of 1 ml. 0·1 *M* versene *p*H 7·4 to the reaction mixture slows the reaction to negligible proportions. Colour development increases with *p*H as reported by Lambert for resorcinol oxidation. Under these conditions the colour increases linearly with the incubation time and linearly with the amount of copper added (respective limits studied, 1 hr. and 2 μgm. copper). After correction for blank colour-formation in the absence of added copper, a concentration of $1{\cdot}8 \times 10^{-7}$ *M* copper in the incubation mixture gave an extinction at 460 mμ of 0·1 (path length, 1 cm.) after a 20 min. incubation.

The effects of metal ions commonly occurring in biological extracts was investigated to see the extent of interference in the copper-catalysed orcinol oxidation. The following metal ions had no influence on the reaction when present in large final concentrations (at least 40-fold excess over copper) : sodium, potassium, zinc, calcium, magnesium, aluminium and ferrous ions. Cobaltous and manganous ions stimulated the orcinol oxidation in a similar manner to copper ions ; ferric ions and chromic ions inhibited the reaction. Comparative effects of these metal ions are shown in Fig. 2. The effects of several of the above metal ions on the oxidation of alkaline orcinol are different from their effects on resorcinol oxidation[2,3]. It is apparent from the results in Fig. 2 that unless the biological extracts are abnormally rich in manganese or ferric ions compared with copper, the orcinol colour will be largely the result of copper-induced oxidation and, as such, will reflect the copper content of the extracts. In general, the manganese content of biological extracts is much less than the copper content[4], but ferric ion inhibition is possible, particularly if appreciable blood is present. As was to be expected, the copper-orcinol reaction

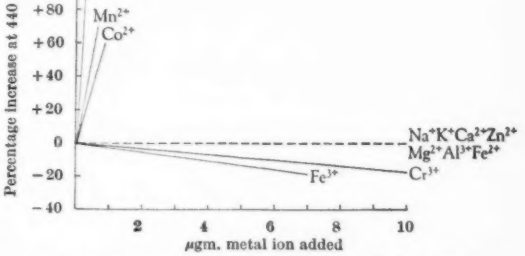


Fig. 2. Effect of various metal ions on the alkaline oxidation of orcinol. The ordinate expresses the percentage increase in colour (at 440 mμ) produced by the addition of metal ions compared with the colour produced in the absence of added metal; the abscissa is the amount of metal ion added in μgm. Other conditions as in the text. The results are representative of various incubation times from 10 min. to 3 hr. but are suitable for comparisons, that is, weight for weight; copper is approx. three times as effective as manganese and about six times as effective as cobalt

was inhibited by traces of protein or amino-acids which bind the copper in an unreactive form. Reducing and oxidizing reagents (for example, vitamin C and hydrogen peroxide) also interfere. Thus if copper estimations by either the copper-orcinol or resorcinol procedures are to be carried out on biological samples, the tissue must be ashed prior to copper estimation. With certain high protein/high fat samples (milk, for example) the copper orcinol procedure becomes unsuited for the estimation of low contents of copper due to the high blank values consequent on ashing. It would appear from the foregoing results and from previous work on resorcinol[2,3] that the catalytic action of copper on phenol oxidation can be used for estimating copper in biological samples providing that the ashing procedure does not require large amounts of digestion material. In other words, the use of the method would seem to lie with samples relatively enriched in copper compared to protein/fat.

Much of this work was done during the tenure of a Beit Memorial fellowship held at University College, London. I am grateful to Prof. C. Rimington and Dr. A. L. Greenbaum for helpful comments.

[1] Engle, R. L., and Dempsey, E. W., *J. Histochem.*, **2**, 9 (1954).
[2] Lambert, R. H., *Anal. Chem.*, **24**, 869 (1952).
[3] Shapiro, M. Ya., *Zhur. Anal. Khim.*, **4**, 199 (1949).
[4] Spector, W. S., in *Handbook of Biological Data* (W. B. Saunders Co., London, 1956).

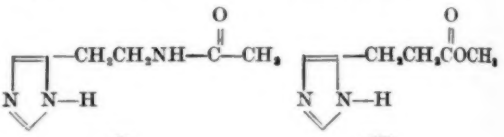
PARTICIPATION OF IMIDAZOLE IN INTRAMOLECULAR HYDROGEN BONDING

By Prof. A. LUKTON
Brooklyn College, Brooklyn, New York

IT is generally believed that an understanding of proximity effects of neighbouring groups functioning in concerted action on an enzyme surface will lead to an explanation of the great disparity between the rates of catalysis of enzymes and of their model systems[1]. Precise determination of the particular role of groups such as imidazole in enzyme catalysis thus requires that its intramolecular structural relationships be established. In this article, evidence for intramolecular hydrogen bonding involving the imidazole ring is presented.

The assignment of the imidazole infra-red absorption bands were made by Otting[2] and include strong bands at 2,600–3,200 cm.$^{-1}$ and at 3,475 cm.$^{-1}$. He demonstrated that the strong NH (unbonded) absorption at 3,475 cm.$^{-1}$ decreased in intensity but not in frequency with increasing concentration in chloroform solution. At the same time the hydrogen bonded NH bands in the region 2,600–3,200 cm.$^{-1}$ increased in intensity, indicating an increase in intermolecular hydrogen bonding. Recently, Anderson *et al.*[3] established the relationship between the absorption intensity change and the length of the linear imidazole oligomers as effected by the imidazole concentration in carbon tetrachloride solution. Such absorption changes should be useful in determining the existence of intramolecular hydrogen bonding in compounds which contain the imidazole ring. In such a structure no imidazole absorption should occur in the 2,600–3,200 cm.$^{-1}$ region since imidazole participation in intramolecular hydrogen bonding would preclude the possibility of its participation in intermolecular bonding in dilute, non-hydrogen bonding solvents.

Acetyl histamine (I) and methyl dihydrourocanate (II) were studied to examine this premise.



(I) (II)

In (I) intramolecular hydrogen bonding could involve: the hydrogen linked to the ring pyrrole nitrogen and the carbonyl oxygen of the amide

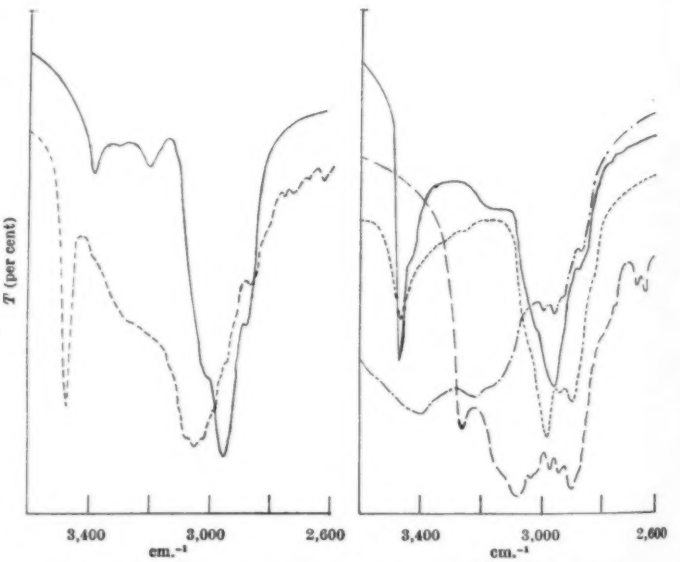


Fig. 1. The infra-red absorption spectra of: left, 1,4-methylhistamine in $CHCl_3$ (——), imidazole in $CHCl_3$ (– – –); right, acetylhistamine in $CHCl_3$ (– – –), acetylhistamine in KBr (— — —), methyl dihydrourocanate in $CHCl_3$ (——), methyl dihydrourocanate in KBr (— – —)

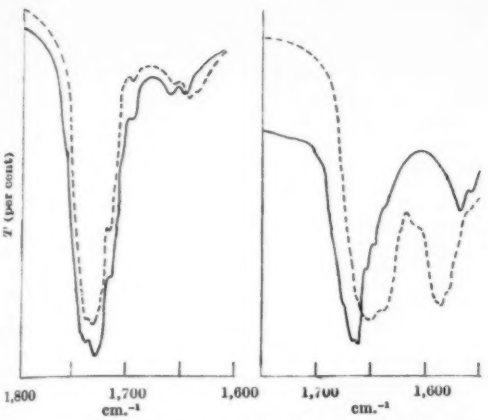
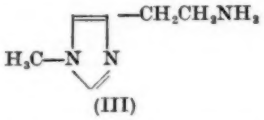


Fig. 2. The infra-red absorption spectra of : left, methyl dihydrourocanate in $CHCl_3$ (——), methyl dihydrourocanate in KBr (– – –); right, acetylhistamine in $CHCl_3$ (——), acetylhistamine KBr (– – –)

linkage ; or, the ring pyridine nitrogen and the imino hydrogen of the amide group. Hydrogen bonding in (II) could result only in the bonding of the hydrogen of the pyrrole nitrogen and the carbonyl of the ester group.

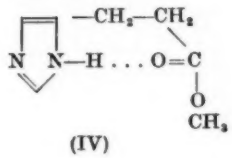
The intra-red spectra of acetyl histamine, methyl dihydrourocanate, imidazole and 1,4-methylhistamine (1-methyl-4-(β-amino-ethyl)-imidazole) in the region 2,600–3,600 cm.$^{-1}$ and 1,600–1,800 cm.$^{-1}$ are given in Figs. 1 and 2. The 2,900–3,100 cm.$^{-1}$ region of the methyl dihydrourocanate and acetyl histamine spectra is complicated by the coincident absorption of CH, CH_2 and CH_3. The contribution of this absorption, however, was determined in the absence of imidazole NH (bonded) absorption by examining the spectrum of 1.4 methylhistamine, structure III, in chloroform solution.



(III)

As shown in Fig. 1, there is a strong absorption band at 2,967 cm.$^{-1}$, (CH_3), a weak band at 2,878 cm.$^{-1}$ and a shoulder at 3,007 cm.$^{-1}$. The two weak absorption bands at 3.373 and 3,310 cm.$^{-1}$ are due to the NH vibration of the NH_2 group[4].

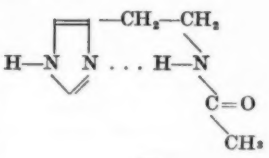
Examination of the methyl dihydrourocanate spectrum in chloroform solution reveals a spectrum similar to that of 1,4 methylhistamine with a strong band at 2,957 cm.$^{-1}$ and a weaker band at 2,878 cm.$^{-1}$. Furthermore, it is unlike the imidazole spectrum in chloroform solution since the strong imidazole band at 3,048 cm.$^{-1}$ is completely absent and leads to the postulate that intermolecular hydrogen bonding does not occur due to the existence of the internally bonded structure (IV).



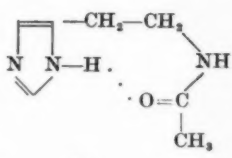
(IV)

The behaviour of the absorption band of the ester carbonyl stretching vibration in solution and in the solid state lends support for this structure. Compounds such as *N*-methylacetamide in the solid state in which intermolecular hydrogen bonding occurs, absorb at a lower frequency (C=O bonded, 1,655 cm.$^{-1}$) than in their dilute solutions (C=O unbonded, 1,695 cm.$^{-1}$)[5,6]. However, methyldihydrourocanate absorbs at 1,730 cm.$^{-1}$ in dilute and concentrated chloroform solutions and in the solid state, Fig. 2. The expected frequency shift did not occur since the hydrogen bond in this compound, whether inter- or intra-molecular, involves the same atoms. That intermolecular hydrogen bonding occurs in the solid state between NH (imidazole) and C=O (ester) is indicated in its spectrum in Fig. 1. Here, broad absorption bands exist at 3,390 and 3,216 cm.$^{-1}$. This is characteristic of intermolecular bonded hydrogen in amides, although the amide bonds are at 3,300, 3,275 and 3,070 cm.$^{-1}$ (ref. 6).

The absorption spectrum of acetylhistamine in chloroform solution is also similar to that of 1,4 methylhistamine. It has a strong band at 2,986 cm.$^{-1}$, a weak band at 2,900 cm.$^{-1}$ and it lacks the bonded NH (imidazole) band at 3,048 cm.$^{-1}$. The absence of bonded NH (imidazole) absorption in this region indicates intramolecular hydrogen bonding which could result from two possible structures (V) and (VI).

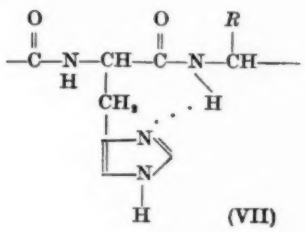


(V)

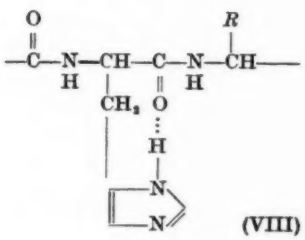


(VI)

Structure (V) is favoured because it consists of a six-membered hydrogen-bonded ring which is considerably more stable than the eight-membered ring of structure (VI)[7]. Also, there is a frequency shift in the carbonyl absorption from 1,660 to 1,650 cm.$^{-1}$ in going from chloroform solution to the solid state (Fig. 2). As would be expected, the shift is small compared with the shift observed in completely unbonded and bonded amides.



(VII)



(VIII)

The smaller frequency shift is explained on the basis of a change from intra-molecular hydrogen bonding V to intermolecular hydrogen bonding in which the NH (amide) and C=O (amide) participate. Absorption at 3,266, 3,083 and 3,035 cm.$^{-1}$ and characteristic of NH . . . O=C absorption in amides are observed in solid acetyl histamine (Fig. 1). An analogous structure to that proposed by Mizushima[5] for *N*-methylacetamide is suggested.

Several interesting consequences with respect to naturally occurring substances follow from these considerations. In so far as imidazole may participate in protein hydrogen bonding, two configurations would be possible (besides those which may involve bonding with other amino-acid residues).

Both structures contain seven-membered rings: however, structure (VIII) would be favoured. If such structures do exist in proteins, they might help provide an explanation for marked differences in reactivity of various histidine residues such as exist in ribonuclease[9].

Intramolecular hydrogen bonding in the dipeptide carnosine (β-alanyl-histidine) may exert an effect in facilitating the dissociation of the amide imino hydrogen atom during the formation of the copper carnosine chelate[10,11], also it may contribute to the base strength of the imidazole of carnosine (pK (Im) = 6·83) which is a considerably stronger base than that in histidine (pK (Im) = 6·00)[12].

I am indebted to Dr. R. W. Schayer, Merck Institute for Therapeutic Research, Rahway, New Jersey, for supplying the 1,4 methylhistamine.

This investigation was supported by a research grant from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service grant No. *A*-3066.

Research on this project was conducted at Adelphi College, Garden City, N.Y.

[1] Koshland, jun., D. E., in *Advances in Enzymology*, 22 (Interscience Publishers, Inc., New York, 1960).
[2] Otting, W., *Chem. Ber.*, **89**, 2887 (1956).
[3] Anderson, D. M., Duncan, J. C., and Rossotti, F. J. C., *J. Chem. Soc.*, 2185 (1961).
[4] Bellamy, L. J., *The Infra-Red Spectra of Complex Molecules* (John Wiley and Sons, New York, 1954).
[5] Mizushima, S., *et al.*, *J. Amer. Chem. Soc.*, **72**, 3490 (1950).
[6] Sutherland, G. B. B. M., in *Advances in Protein Chemistry*, **7** (Academic Press, New York, 1952).
[7] Pimental, G. C., and McClellan, A. L., *The Hydrogen Bond* (W. H. Freeman and Co., San Francisco, California, 1960).
[8] Mizushima, S., in *Advances in Protein Chemistry*, **9** (Academic Press, Inc., New York, 1954).
[9] Stein, W. D., and Bernard, E. A., *J. Mol. Biol.*, **1**, 350 (1959). Bernard, E. A., and Stein, W. D., *ibid*, **1**, 339 (1959). Gundlach, H. G., Stein, W. H., and Moore, S., *J. Biol. Chem.*, **234**, 1754 (1959).
[10] Dobbie, H., and Kermack, O., *Biochem. J.*, **59**, 254 (1955).
[11] Lukton, A., and Sisti, A., *J. Org. Chem.*, **26**, 617 (1961).
[12] Cohn, E. J., and Edsall, J. T., *Proteins, Amino Acids and Peptides* (Reinhold Pub. Co., New York, 1943).

PROPERTIES OF SOME COPPER COMPLEXES

Five-Covalent Copper(II) Complexes containing 1,10-Phenanthroline and 2,2′-Bipyridyl

THIS preliminary communication reports the preparation and properties of an extensive series of five-covalent copper(II) complexes by the reaction of *bis*(1,10-phenanthroline)copper(II) and *bis*(2,2′-bipyridyl)copper(II) perchlorates with various neutral and negatively charged ligands.

The addition of halide ions (Cl^-, Br^- and I^-) to nitrobenzene or nitromethane solutions of $[Cu(phen)_2](ClO_4)_2$ leads to the formation of variously coloured solutions (blue, green and brown respectively) containing monohalogeno-*bis*(1,10-phenanthroline)-copper(II) ions due to the reaction:

$$[Cu(phen)_2]^{2+} + X^- \rightarrow [Cu(phen)_2X]^+$$

This 1 : 1 reaction has been confirmed both by conductometric titrations and spectrophotometric continuous variation studies. Also, by working in aqueous acetone solutions, a series of coloured crystalline compounds of the type $[Cu(phen)_2X]ClO_4$ (where $X = Cl^-$, Br^-, I^-, SCN^-, NO_2^-, $HCOO^-$, CH_3COO^- and $C_6H_5COO^-$) has been isolated. These compounds possess molecular conductivities in the ranges 27–29 mho and 87–90 mho at 25° in 10^{-3} M nitrobenzene and nitromethane solutions respectively. These values are comparable with those of other uni-univalent electrolytes in these solvents[1] and practically half those of the diperchlorate $[Cu(phen)_2](ClO_4)_2$ (Table 1). The *bis*(2,2′-bipyridyl) compound $[Cu(bipy)_2](ClO_4)_2$ also reacts readily with halide ions in the above two solvents and compounds of the type $[Cu(bipy)_2X]ClO_4$ (where $X = Cl^-$, Br^-, I^- and SCN^-) have also been isolated. These compounds possess similar conductivities to their phenanthroline analogues (see Table 1).

Table 1. MOLECULAR CONDUCTIVITIES AND MAGNETIC MOMENTS OF VARIOUS 1,10-PHENANTHROLINE AND 2,2′-BIPYRIDYL COMPLEXES OF BIVALENT COPPER

Compound	Molecular conductances (mho) in 10^{-3} M solutions at 25°		Magnetic moment per Cu atom in B.M.
	$C_6H_5NO_2$	CH_3NO_2	
$[Cu(phen)_2](ClO_4)_2$	43·1	176	1·94
$[Cu(phen)_2](ClO_4)_2$			1·91
$[Cu(phen)_2X]ClO_4$ (X = Cl^-, Br^-, I^-, SCN^- NO_2^-, $HCOO^-$, CH_3COO^- and $C_6H_5COO^-$)	27–29	87–91	1·83–1·96
$[Cu(phen)_2X]X$ (Cl^-, Br^-, I^-, SCN^-, $HCOO^-$ and CH_3COO^-)	24–27	82–93	1·86–1·97
$[Cu(phen)_2L](ClO_4)_2$ (L = NH_3 and C_5H_5N)		176–199	1·84–2·00
$[Cu(bipy)_2](ClO_4)_2$	54	186	2·03
$[Cu(bipy)_2](ClO_4)_2$	44·5	181	1·93
$[Cu(bipy)_2X]ClO_4$ (X = Cl^-, Br^-, I^-, and SCN^-)	28–31	89–91	1·89–1·97
$[Cu(bipy)_2X]X$ (X = Cl^-, Br^-, I^-, and SCN^-)	11–30	79–85	1·81–1·96
$[Cu(bipy)_2NH_3](ClO_4)_2$		199	1·92
$[Cu(bipy)Cl_2]$		~1	1·96

Attempts to prepare a monocyano-derivative of the $[Cu(phen)_2]^{2+}$ ion lead to the isolation of the cyano-bridged complex:

$$[(phen)_2Cu - C \equiv N - Cu(phen)_2](ClO_4)_3.H_2O$$

in which the cyanide group is linking two $[Cu(phen)_2]^{2+}$ ions.

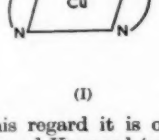
The *bis*-complexes $Cu(phen)_2X_2.nH_2O$ (X = Cl^-, n = 3·5; X = Br^- and I^-, n = 1; X = SCN^-, n = 0; and X = $HCOO^-$ and CH_3COO^-, n = 6) and $Cu(bipy)_2X_2.nH_2O$ (X = Cl^-, n = 6; X = Br^-, n = 2; X = SCN^- or I^-, n = 0) were also prepared and their properties examined. These complexes are also uni-univalent electrolytes in nitromethane solution (Table 1) and contain the $[Cu(chelate)_2X]^+$ ion. In the more weakly polar solvent nitrobenzene they undergo some re-arrangement due to the reaction:

$$[Cu(chelate)_2X]^+ + X^- \rightleftharpoons [Cu(chelate)X_2] + chelate$$

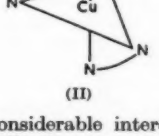
This equilibrium, which is independent of concentration, has been studied in detail by means of accurate conductivity measurements over a concentration range and will be reported later[2]. Mono-complexes of the type $[Cu(bipy)X_2]$ (where X = Cl^-, Br^- and SCN^-) were prepared and examined during the course of this work. For example, the dichloro-complex $[Cu(bipy)Cl_2]$, which is readily obtained by adding the ligand to excess copper(II) chloride in ethanol, is virtually a non-electrolyte in nitromethane solution (Table 1).

The reaction of neutral ligands with $[Cu(phen)_2]^{2+}$ and $[Cu(bipy)_2]^{2+}$ has also been studied and the stable mono-ammine complexes $[Cu(chelate)_2(NH_3)](ClO_4)_2$ were obtained from aqueous ammonia solutions. The *bis*(1,10-phenanthroline) complex also forms the mono-pyridine complex $[Cu(phen)_2(py)](ClO_4)_2$ as well as the aquo-derivative $[Cu(phen)_2(H_2O)](ClO_4)_2$. These compounds function as di-univalent electrolytes in nitromethane solution (see Table 1).

There is a number of possible structures for the five-covalent ions $[Cu(chelate)_2X]^+$ and $[Cu(chelate)_2L]^{2+}$ (where X is a negative ion and L is a neutral ligand). They could, for example, possess the square-pyramidal arrangement I, or the trigonal bipyramidal one represented in II.



(I)



(II)

In this regard it is of considerable interest that Barclay and Kennard (see following communication) have shown that the structure of iodo-*bis*(2,2′-bipyridyl)copper(II) iodide contains an approximately trigonal bipyramidal arrangement about the copper atom in the $[Cu(bipy)_2I]^+$ ion. This type of arrangement confers the possibility of mirror-image isomerism on the copper atom and the unit cell contains both isomers. The X-ray data reveal that the Cu – N and Cu – I distances correspond to normal covalent bond distances whereas the stereochemistry of bivalent copper is usually such that the copper atom is surrounded by four atoms at normal covalent bond distances in the plane with one or two more elongated bonds normal to the plane.

The magnetic moments of the 5-covalent complexes in the table all fall in the range 1·8–2·0 B.M. These values are similar to those of copper(II) complexes in general.

The magnetic moments of $[Cu(phen)_2Br]ClO_4$ and $[Cu(phen)_2Br]Br$ have been reported previously as being essentially independent of temperature over the range 90–300° K. (ref. 3).

C. M. Harris
T. N. Lockyer
Hanneke Waterman

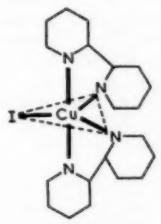
The Trigonal-Bipyramidal Configuration of the Iodo *Bis*(2-2′-Bipyridyl)Copper(II) Ion

The compounds $Cu(dipy)_2X_2$ and $Cu(phen)_2X_2$ formed between 2,2′-bipyridine or *o*-phenanthroline and copper(II) salts have been shown to have co-ordination numbers of five in non-aqueous solutions (see previous communication). The purpose of this communication is to record preliminary results of an X-ray structure determination of one of them, *bis*(2,2′-bipyridyl)copper(II) iodide.

The lustrous brown crystals are triclinic (a = 10·66 ± 0·01; b = 14·37 ± 0·01; c = 7·44 ± 0·01 Å.; α = 93 ± 1°; β = 101 ± 1°; γ = 108 ± 1°). There are two molecules of composition $Cu(C_{10}H_8N_2)_2I_2$ in the unit cell ($d_{meas.}$ = 1·99; $d_{calc.}$ = 1·98 gm. cm.$^{-3}$). The centro-symmetric space group, $P_{\bar{1}}$, was assumed.

One-thousand-four-hundred independent intensities were estimated visually from equi-inclination Weissenberg photographs taken with copper radiation. The co-ordinates of the atoms were determined from three-dimensional Patterson and Fourier syntheses. With a disagreement index ($\Sigma||F_{obs.}| - |F_{calc.}||/\Sigma|F_{obs.}|$) of 0·18, the estimated standard deviations of the atomic positions were I, 0·003; Cu, 0·005; N, 0·03; C, 0·05 Å. The structure is being refined further in an attempt to locate the light atoms more precisely. However, it is quite clear that the copper atom has an unusual stereochemistry.

The compound contains a positively charged iodo-*bis*(bipyridyl)copper(II) ion and a negatively charged iodide ion. The copper atom is surrounded by four nitrogen atoms (at a distance of 2·02 Å.) and an iodine atom (at 2·71 Å.) at the corners of a distorted trigonal bipyramid. The iodine atom and two nitrogen atoms (from different bipyridine molecules) are in the same plane as the copper atom. The other two nitrogen atoms lie on a line (passing through the copper atom) which makes an angle of about 9° with the normal to this plane. The arrangement is shown in III:



(III)

All five atoms are at normal covalent bond distances from the copper atom. The trigonal bipyramidal configuration for the copper atom differs in this respect from the usual stereochemistry of bivalent copper which involves four bonds of normal covalent lengths with a fifth, and sometimes a sixth, at a greater distance. As the iodo-*bis*(2,2′-bipyridyl)-copper(II) ion possesses neither a plane nor a centre of symmetry, the two ions in the unit cell are related to one another as optical isomers.

Terpyridylcopper(II) chloride dihydrate is isomorphous with terpyridylzinc chloride, which has a similar trigonal bipyramidal configuration about the metal atom[4].

G. A. Barclay
C. H. L. Kennard

School of Chemistry,
University of New South Wales,
Broadway,
New South Wales.

[1] Harris, C. M., and Nyholm, R. S., *J. Chem. Soc.*, 4375 (1956). Harris, C. M., *ibid.*, 682 (1959). Harris, C. M., Nyholm, R. S., and Phillips, D. J., *ibid.*, 4379 (1960).
[2] Barker, N. S., thesis, University of New South Wales (1960).
[3] Figgis, B. N., and Harris, C. M., *J. Chem. Soc.*, 855 (1959).
[4] Corbridge, D. E. C., and Cox, E. G., *J. Chem. Soc.*, 594 (1956).

THERMAL REACTION BETWEEN TWO LIGHT REACTIONS IN PHOTOSYNTHESIS

By Prof. C. P. WHITTINGHAM and P. M. BISHOP
Botany Department, Queen Mary College, London, E.1

ALLEN and Franck[1] showed with the alga *Scenedesmus* that a single flash of less than 1 msec. in duration resulted in no detectable oxygen evolution from photosynthesis. If two flashes were given, separated by a suitable dark interval, a measurable yield was obtained. Alternatively an increased yield was obtained when a single flash was superimposed on a continuous background of low intensity. Whittingham and Brown[2] confirmed and extended these observations with the alga *Ankistrodesmus*. They interpreted their results in terms of two photochemical reactions, the first of which was thought to activate an essential catalyst of photosynthesis, the second being concerned in photosynthesis directly.

More recent work has suggested the existence of two photochemical reactions in the photosynthetic process. This has been postulated to explain the enhancement (or 'Emerson') effect on photosynthesis obtained with a number of algæ excited with light of two different wave-lengths[3]. Govindjee *et al.*[4] showed that the enhancement effect was also observed for the photoreduction of quinone by *Chlorella* (Hill reaction). Duysens *et al.*[5] showed with *Porphyridium* that light, absorbed by the phycobilins, preferentially resulted in the reduction of cytochrome *f*, whereas light at 680 mμ absorbed by chlorophyll predominantly oxidized the cytochrome. Losada *et al.*[6] independently suggested, on the basis of work with isolated chloroplasts, that one pigment system was concerned in the liberation of oxygen from water and the transfer of an electron to an acceptor and a second pigment system was concerned with the acceptance of the electrons from this first acceptor. They produced evidence to suggest that the second system was that in which photophosphorylation was concerned and deduced that cytochromes were probably concerned here.

Our earlier observations with flash illumination can also be interpreted in terms of a mechanism involving two light steps. In this article are further results on the effects of flash illumination with isolated chloroplasts which further clarify the mechanism involved.

Ankistrodesmus was cultured in the manner described in ref. 2. Chloroplasts were isolated from *Beta vulgaris* (perpetual spinach, Thompson and Morgan, Ltd.) grown either in the field or in a greenhouse without close control of growth conditions. The chloroplasts were isolated at 0° C. by grinding in a medium containing 0·35 *M* sodium chloride, 0·17 *M* *tris* and 0·03 *M* $MgCl_2 6H_2O$ at *p*H 7·4, washed once in the grinding medium, and then re-suspended in the same medium diluted one in ten. The chloroplasts were kept on ice throughout the period before measurement. A reaction mixture containing 10 μmoles $Fe_3(CN)_6$, 6 μmoles $MgCl_2 6H_2O$, 70 μmoles sodium chloride and 40 μmoles *tris* at *p*H 7·4 was made up to 2·5 ml., and when required 2 μmoles adenosine diphosphate and 40 μmoles inorganic phosphate added. For the purposes of uncoupling phosphorylation 10^{-2} *M* ammonium chloride was added. In other experiments ferricyanide was replaced by 0·5 μmole phenol-indo 2,6-dichlorophenol. Chloroplasts prepared in this way and kept at 0° C. were found reasonably stable during the period required for the experiment. Measurements could not begin for 40 min. after preparation of the chloroplasts, since it was necessary to remove oxygen from the reaction mixture. Two types of light flash were used: a 'Mazda' discharge tube *FA4* giving a flash of approximately 0·2 msec. duration (short flash) and a Philips photoflux bulb *PF60* approximately 30 msec. (long flash). All other conditions of measurement were as described before except that the reaction vessel was maintained at 4° C.

Whittingham and Brown found that the maximum yield from a pair of flashes was obtained with *Ankistrodesmus* when the short flash preceded the long flash by a dark interval of 0·7 sec. at 25° C. Some influence of the short flash on the yield from the long flash was detectable even with a dark separation of up to 15 sec. Further work has shown that when the temperature was reduced to 4° C. the yield from the long flash alone was much decreased; but the absolute maximal yield for a long flash preceded by a short flash was of the same order of magnitude as the maximum yield for the same two flashes at 20° C., except that the time between flashes for maximal yield was now 16 sec. (Fig. 1). A similar value was found for the time of separation of the two flashes for optimal production of oxygen from ferricyanide by chloroplasts isolated from spinach beet. It is suggested that a dark thermal reaction, interposed between the two photochemical processes, is necessary for some conversion of the products of process 1 to become the reactants for process 2. The duration of the short flash is so brief that during the flash none of the product of the first reaction has been converted by the second. Witt *et al.*[7] have shown that the absorption changes in *Chlorella* at −150° C. indicate that only one photochemical process predominates at low temperature. Experiments reported here indicate a significant effect of temperature even within the physiological range. The results in Fig. 1, which compare the yield for two flashes as a function of their separation in time at 4° and 20° C., suggest that the thermal reaction proceeds more slowly but that its product has a greater half-life at the lower temperature. This is consistent with the observations of Emerson *et al.*[8], who showed that at lower temperatures photosynthetic efficiency was increased at longer wave-lengths in the far red consistent with an increased life-time at low temperature of the product of a reaction characteristic of shorter wave-length absorption.

When phenol-indo 2,6-dichlorophenol was used instead of ferricyanide, the optimal separation between flashes was considerably shorter, being less than 5 sec. at 4° C. This indicates that dichlorophenol indophenol can result in production of oxygen without

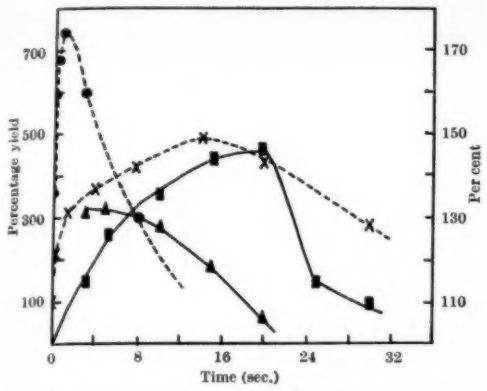


Fig. 1. Relationship between the combined yield of oxygen for a short and long flash as a function of the dark time separating the two flashes. The yield is expressed as a percentage of the sum of the yields from the two flashes given simultaneously. ●, *Ankistrodesmus* cells in bicarbonate buffer at 20° C.; ■, Isolated beet chloroplasts with ferricyanide at 4° C.; ▲, Isolate chloroplasts with ferricyanide and ammonium chloride at 4° C. (all right-hand ordinate); ×, *Ankistrodesmus* cells in bicarbonate at 4° C. (left-hand ordinate)

proceeding through the same thermal reaction as is required for ferricyanide reduction. Addition of catalytic amounts of indophenol (0·01 μmole) in the presence of ferricyanide (10 μmoles) changed the optimal time between flashes from that characteristic of ferricyanide alone to that characteristic of dye alone. This is consistent with the results observed in continuous illumination[6], which Losada *et al.* interpreted as indicating that reduction of dye could take place as a result of only one photochemical reaction.

The difference in time of separation between flashes for optimal yield for ferricyanide and dye may be related to the fact that reduction of ferricyanide is accompanied by phosphorylation whereas that of dye is not. Addition of adenosine diphosphate and inorganic phosphate to a system containing ferricyanide did not result in any significant change in the time of flash separation for optimal yield, but in the presence of ammonium chloride, which has been shown to uncouple the reduction of ferricyanide from phosphorylation[9], the separation in time was significantly shortened. It became of the same order of magnitude as that in the presence of indophenol alone.

The two types of flash used differ in spectral composition. Further experiments, using monochromatic light flashes, have shown that with a long flash of 692 mμ a maximal effect is obtained with weak continuous illumination of 674 mμ. A full investigation of the whole action spectrum is now in progress.

These results are consistent with a mechanism of photosynthesis involving two light reactions separated by a thermal dark process. The thermal dark process is apparently accelerated when phosphorylation does not accompany transfer of electrons. Phosphorylation may be concerned directly in the thermal stage as previously suggested or if it accompanies the second photochemical step the rate of transfer of electrons from process 1 to process 2 must be slower in the presence of phosphorylation than in its absence.

We are grateful to the Royal Society for a Government grant-in-aid of this investigation. One of us (P. M. B.) holds a postgraduate studentship of the University of London. We are also grateful for a grant from the Central Research Fund of the University of London.

1 Allen, F. L., and Franck, J., *Arch. Biophys. Biochem.*, **58**, 124 (1955).
2 Whittingham, C. P., and Brown, A. H., *J. Exp. Bot.*, **9**, 311 (1958).
3 Myers, J., and French, C. S., *J. Gen. Physiol.*, **43**, 723 (1960).
4 Govindjee, R., Thomas, J. B., and Rabinowitch, E. R., *Science*, **132**, 421 (1960).
5 Duysens, L. M. N., Amesz, J., and Kamf, B. M., *Nature*, **190**, 510 (1961).
6 Losada, M., Whatley, F. R., and Arnon, D. I., *Nature*, **190**, 606 (1961).
7 Witt, H. T., Müller, A., and Rumberg, B., *Nature*, **191**, 194 (1961).
8 Emerson, R., Chalmers, R., and Cederstrand, C., *Proc. U.S. Nat. Acad. Sci.* (Wash.), **43**, 133 (1957).
9 Krogmann, D. W., Jagendorf, A., and Avron, M., *Plant Physiol.*, **34**, 272 (1959).

SULPHATE TRANSPORT AND METABOLISM BY *DESULPHOVIBRIO DESULPHURICANS*

By Prof. C. FURUSAKA*
McGill-Montreal General Hospital Research Institute

LITTLEWOOD and Postgate[1] stated that the micro-organism *Desulphovibrio desulphuricans* is not freely permeable to anions such as sulphate and chloride. They suggested that sulphate reduction occurs either outside the osmotic barrier or that the penetration of sulphate into the organism is regulated in such a manner that at equilibrium the ratio of the internal concentration of sulphate ions to the external concentration is so low that the analytical procedure used in their work could not detect penetration of the ion even at high external sulphate concentrations. Skarzynski and Ostrowski[2] claimed that *Thiobacillus thioparus* takes up only the outer sulphur atom of thiosulphate, leaving the inner sulphur atom of thiosulphate outside the cell as sulphate ion.

These two papers give an impression that the inorganic sulphur compounds are metabolized on the surface of the cells, even though sulphate and thiosulphate are the main energy-yielding substrate for these organisms.

It was of interest to elucidate whether these organisms, which utilize inorganic sulphur compounds, actually metabolize their substrates at the surface of the cell outside the osmotic barrier.

This communication is concerned with the problem of sulphate accumulation and metabolism in the cells of a strain of *Desulphovibrio*, which was isolated from a muck paddy field in Japan: this organism plays a very important part in the reduction of sulphate to sulphide in the soil.

The transport of sulphate into the cells was examined using a moderately thick suspension of cells (about 27·5 mgm. dry weight of cells was added to each reaction mixture; this corresponded to about 0·1 ml. of packed cells per 2 ml. of reaction mixture), as it was found that the ordinary thick suspension

*Present address: Institute for Agricultural Research, Tohoku University, Sendai, Japan.

method[3] was not suitable for substances which are metabolized very quickly by the cell. The cells were incubated with radioactive sulphate in Warburg vessels at 37° C. in a *tris* buffer of *p*H 7·2, using hydrogen in the gas phase. After incubation, the contents of each vessel were rapidly cooled with ice and then centrifuged at 18,000*g* for 14 min. in a refrigerated centrifuge. The supernatant was removed by decantation for the estimation of sulphate and sulphide. The packed cells were rinsed three times with ice-cooled water very quickly to remove radioactive sulphate adhering to the wall of the centrifuge tube and to the surface of the packed cells.

For the estimation of sulphate in the supernatant, an aliquot of the supernatant was placed in a test-tube and to it was added one drop of *N* hydrochloric acid. Then the test-tube was put in a boiling water-bath for 2 min. so as to eliminate hydrogen sulphide. The solution was diluted ten times with distilled water; 0·5 ml. of the diluted solution was placed on an aluminium plate and dried by an infra-red lamp. The radioactivity on the plate was measured by a Geiger–Müller counter and expressed as the amount of sulphate in the reaction mixture.

For the estimation of sulphide in the supernatant, 1 ml. of the original supernatant was strongly acidified by hydrochloric acid in a distillation apparatus and the hydrogen sulphide evolved was driven into a zinc acetate solution, contained in a volumetric flask, by bubbling nitrogen gas through the apparatus for 1 hr. The sulphide content of the zinc acetate solution was estimated by St. Lorant's methylene blue method[4]. The radioactivity of sulphide-sulphur was estimated by measuring the radioactivity of methylene blue adsorbed on carbon powder.

The packed cells were extracted with 10 ml. of 5 per cent trichloroacetic acid solution at 0° C. An aliquot of the extract was dried on a steam-bath so as to drive off the hydrogen sulphide and almost all the trichloroacetic acid; then the residual organic matter in it was oxidized completely by hydrogen peroxide. The residues were dissolved in a known amount of water and applied to the estimation of sulphate in the packed cells. For this estimation an aliquot of the solution thus prepared was put on an aluminium plate and dried. The radioactivity on the plate was measured and it was expressed as the amount of sulphate in the packed cells.

For the estimation of sulphide in the packed cells, they were transferred into the distillation vessel using about 2 ml. of water and the suspension strongly acidified by hydrochloric acid. The hydrogen sulphide thus evolved was treated by the same procedure as was the supernatant for the estimation of the radioactivity of the sulphide-sulphur.

In this article, the term 'transport' will be used for the description of inflow and outflow. It should also be mentioned that the term 'accumulation' will be used to indicate the result of both transport and reduction.

A typical result of sulphate accumulation in the cells is shown in Fig. 1*a*. The concentration of sulphate in the packed cells was found to be higher than that of the medium at a certain period of the reaction. At the peak of this accumulation, the sulphate concentration in the packed cells was calculated to be 145 per cent greater than that of the reaction medium at that time, or 28 per cent greater than the initial sulphate concentration. This indicates that there is a distinct accumulation of sulphate in the cells.

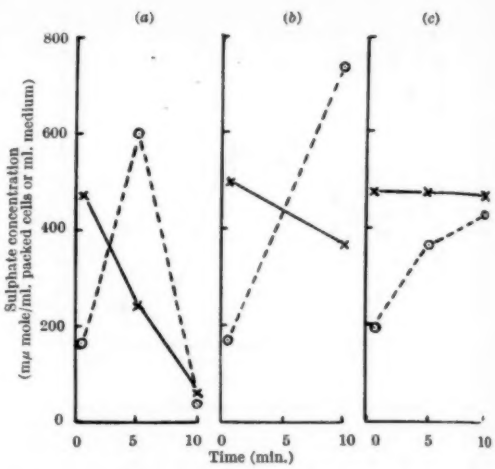


Fig. 1. Sulphate accumulation in the cells. ○ - - - ○, Sulphate concentration in the cells (mμ mole/ml. packed cells). × — ×, Sulphate concentration in the reaction mixture (mμ mole/ml. reaction medium).
(*a*) without selenate; (*b*) with *M*/16,500 selenate; (*c*) with *M*/1,100 or *M*/5,500 selenate.
Each vessel contained 1·0 ml. of *M*/20 *tris*-hydrochloric acid buffer (*p*H 7·2), 0·1 ml. of *M*/1,000 phosphate buffer, 0·1 ml. of magnesium chloride, 0·5 ml. of cell suspension (corresponds to 0·1079 ml. packed cell volume), 0·3 ml. of water, 0·1 ml. of *M*/100 sodium sulphate-^{35}S, and 0·1 ml. of sodium selenate (*M*/750, *M*/250 or *M*/50 corresponding to *b*, *c* or *c*, respectively). In the centre well 0·3 ml. of potassium hydroxide–pyrogallol solution was placed. Total volume was 2·2 ml. Sodium sulphate and sodium selenate were placed in a side-arm and were added to the reaction mixture at zero time

The mean velocity of sulphate transport, which corresponds to the mean velocity of disappearance of sulphate from the medium, and that of accumulation of sulphate in the cells, were calculated from the values obtained after 30 sec. and 5 min. in Fig. 1*a*. The mean velocity of sulphate transport was calculated to be 1,022 mμ mole/ml. packed cells/min., dividing the amount of sulphate which disappeared from the medium (470 − 244 = 226 mμ mole/ml. reaction mixture) by time (4·5 min.) and the volume of packed cells (0·0491 ml.), which corresponds to 1 ml. of the reaction mixture. On the other hand, the mean velocity of sulphate accumulation was calculated to be 96·6 mμ mole/ml. packed cells/min., dividing the amount of sulphate accumulated in the cells (435 mμ mole/ml. packed cells) by the time (4·5 min.). These figures indicate that the sulphate transport is about eleven times more rapid than sulphate accumulation, that is, almost all the sulphate transported into the cells is reduced quickly and only a small part of the sulphate is left in the cells. It must be borne in mind that the amount of sulphate accumulated in the cells is the result of two phenomena: transport and reduction.

It might be possible to show much more accumulation of sulphate in the cells if a way could be found to diminish the velocity of sulphate reduction without affecting the velocity of sulphate transport. As a trial, the hydrogen gas phase of the reaction vessel was replaced by helium. The helium gas was passed through for 2 min., with a consequent reduction of the partial pressure of the hydrogen gas. Much greater accumulation of sulphate in the cells was observed with a helium–hydrogen gas phase than in the pure hydrogen phase, even though both velocities of sulphate reduction and transport were

diminished. It was also observed that sulphate, once accumulated in the cell, passes into the medium rapidly after sulphate reduction has stopped due to the lack of a hydrogen donor.

More detailed experiments were conducted concerning the fate of the sulphide. The concentration of sulphide in the cells was found to be always two to six times higher than that in the medium, even though it was much lower than that of sulphate. The highest concentration observed in the packed cells was about 100 mμ mole/ml. packed cells. Changes in the concentration of sulphide in the cells follow that of sulphate.

Selenate is known as a competitive inhibitor of sulphate reduction[2]. It is also known that selenate competes with sulphate by the formation of a very unstable transient compound with adenosine triphosphate in the presence of sulphurylase[6]. The effect of selenate inhibition was examined at different concentrations. When *M*/16,500 selenate solution was used, a higher accumulation of sulphate in the cells was observed than in the cells without selenate, even though the velocity of sulphate accumulation was lowered (Figs. 1*b* and 1*a*). The inhibition of sulphate transport and sulphate reduction calculated from the Figs. 1*a* and 1*b* were 80 per cent and 85 per cent respectively. These values indicate that sulphate transport and reduction were inhibited by selenate, and the difference between the rates of inhibition of both reactions by selenate was very slight. When the concentration of selenate was high enough to stop the reduction almost completely (95 per cent inhibition of sulphate reduction), no accumulation of sulphate in the cells was observed (Fig. 1*c*). From these results the following possibilities are suggested: either (*a*) sulphate uptake is associated with sulphate reduction, or (*b*) selenate inhibits transport of sulphate directly.

To test whether sulphate transport is associated with sulphate reduction, experiments with oxygen were carried out. Oxygen is a strong inhibitor of the metabolism of this organism. When oxygen is used in the gas phase, the accumulation of sulphate in the cells and the reduction of sulphate are inhibited completely. Further confirmation is seen in the results of diffusion experiments carried out at 0° C. It was observed that 30 per cent of the packed-cell volume took up sulphate within the first 30 sec., but no marked increase of sulphate in the cells was observed in the following 30 min. This figure, 30 per cent of the packed cell volume, corresponds to the inter-cellular volume of packed cells of *Desulphovibrio* (27·3–33·6 per cent as described by Littlewood and Postgate[1]). Even though there was observed a slight increase in permeable volume in 30 min., the final observed value of 36·8 per cent is still far from the percentage of the water content of the packed cells (76·3 per cent w/v). Therefore, little diffusion of sulphate into the cells occurs at 0° C. and it is negligible compared with the velocity of sulphate reduction.

The suggestions of Littlewood and Postgate[1] already cited seem to be only partially true. There was no evidence for the idea that sulphate reduction might occur outside the osmotic barrier of the cell. The main differences between our experimental conditions and those of Littlewood and Postgate are the presence of a hydrogen donor necessary for the reduction of sulphate and the low concentration of sulphate used in the reaction mixture (*M*/2,200). When a higher concentration of sulphate (*M*/220) was used, it was very difficult to observe any distinct accumulation of sulphate in the cell, probably because the concentration of sulphate in the medium was of the same order as the saturation concentration of the cells, the difference between the two being too small to be detected.

My results show that *Desulphovibrio* is not freely permeable to sulphate, this being indicated by the results obtained using the following experimental procedures: (*a*) inhibition by selenate, (*b*) oxygen inhibition of sulphate reduction; and (*c*) diffusion at 0° C. Thus, it must be concluded that: (1) a significant accumulation of sulphate occurs in the cell; (2) sulphate must be metabolized inside the cell; and (3) the transport of sulphate is associated with the reduction of sulphate.

I thank Prof. J. H. Quastel for his valuable advice and interest in this work, and also the U.S. National Science Foundation for financial aid which made this work possible.

[1] Littlewood, D., and Postgate, J. R., *J. Gen. Microbiol.*, **16**, 596 (1957).
[2] Skarzynski, B., and Ostrowski, W., *Nature*, **182**, 930 (1958).
[3] Roberts, R. B., Abelson, P. H., Cowie, D. B., Bolton, E. T., and Britten, R. J., Pub. No. 607, Carnegie Inst., Washington (1959).
[4] St. Lorant, I., *Z. physiol. Chem.*, **185**, 245 (1929).
[5] Postgate, J. R., *Nature*, **164**, 670 (1949); *J. Gen. Microbiol.*, **6**, 128 (1952).
[6] Bandurski, R. S., Wilson, L. G., and Squires, C. L., *J. Amer. Chem. Soc.*, **78**, 6408 (1956).

ANTIGENICITY OF PENICILLIN AND ITS RELATION TO ALBUMIN BINDING

By Dr. TOSHIO TORII, and Dr. YOSHIHI KOHORIUCHI
Hokkaido University Medical School, Sapporo

ANAPHYLACTOID reactions to penicillin are most commonly observed among severe immediate hypersensitivities to antibiotics[1]. Reagin type of antibody was reported to cause these anaphylactoid reactions[2]. However, experimental hypersensitivity to penicillin has not been established by actual demonstration in animals. In this article we present a method to induce active and passive anaphylaxis to penicillin in guinea pigs and discuss the mechanism of antigenic binding of penicillin to albumin.

The method used to induce active anaphylaxis in guinea pigs to penicillin was as follows: Equal volumes of 100,000 u./ml. of penicillin *G* and 10 mgm./ml. of recrystallized egg white albumin or horse serum albumin were incubated at 37° C. for 24 hr. The precipitates obtained by incubation were centrifuged and dissolved in saline to the original volume and adjusted to *p*H 7·6–7·8. For the sensitization 0·1 ml. of this solution of penicillin–albumin complex was injected subcutaneously into guinea pigs twice weekly for a total of 5–10 times. After 3 weeks

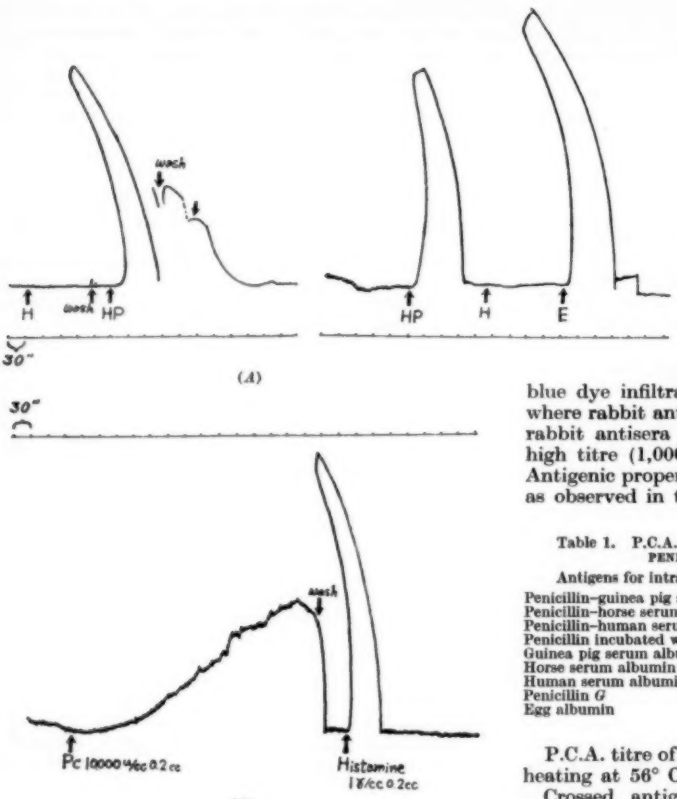


Fig. 1. Schultz-Dale's ileum contraction test in guinea pig sensitized with penicillin–egg albumin complex. (*A*) *H*, horse serum albumin; *HP*, penicillin–horse serum albumin complex; *E*, egg albumin; (*B*), *PC*, penicillin

of incubation, as shown in Fig. 1*A*, the ileum of guinea pig sensitized with penicillin *G*–egg albumin complex showed a typical anaphylactic contraction as a result of contact with penicillin-horse albumin complex and egg albumin, but such contraction was not caused by horse albumin. Penicillin-guinea pig serum albumin complex also can be used for the sensitization and the *in vitro* challenge.

Plain penicillin *G* did not cause the anaphylactic contraction, or an atypical slow contraction was observed as shown in Fig. 1*B*. However, after pre-incubation with penicillin *G* any contraction caused by penicillin *G*–albumin complex was inhibited. By use of the intravenous challenge method positive results were obtained only with penicillin–albumin complex and not with plain penicillin. From these results penicillin appears to be a simple haptene and needs to be bound with albumin in order to gain a complete antigenicity.

Albumins to be combined in this method are not different according to their origins; in other words, they are exchangeable for sensitization and challenge.

Passive Cutaneous Anaphylaxis in Guinea Pigs

Passive cutaneous anaphylaxis (P.C.A.) in guinea pigs by use of Ovary's method[3] was applied to test antigenicity of penicillin and closely related substances.

3 ml. of penicillin–egg albumin complex, 1 ml. of dehydrated lanolin and 8 ml. of liquid paraffin were mixed to make antigen for the sensitization of rabbit. A total 0·2 ml. of the mixture was injected into each foot pad and thereafter 1 ml. subcutaneously once a week for a total of 10 times. One-tenth ml. of arbitrary diluted rabbit antiserum was injected intradermally into shaved abdominal area of guinea pigs. After 5–6 hr., 1 ml. of penicillin–albumin complex (2·5 times dilution of the complex antigen above described) and 1 ml. of 0·5 per cent Evans's blue were injected into jugular vein. Within 30 min. blue dye infiltration was observed in the skin area where rabbit antiserum was previously injected. All rabbit antisera of 8–10 weeks sensitization yielded high titre (1,000 times dilution) of P.C.A. reaction. Antigenic properties of penicillin were simple haptene as observed in the ileum contraction test (Table 1).

Table 1. P.C.A. IN GUINEA PIGS WITH RABBIT ANTIBODY TO PENICILLIN–EGG ALBUMIN COMPLEX

Antigens for intravenous challenge	Results
Penicillin–guinea pig serum albumin complex	+++
Penicillin–horse serum albumin complex	+++
Penicillin–human serum albumin complex	+++
Penicillin incubated with human gamma globulin	—
Guinea pig serum albumin	—
Horse serum albumin	—
Human serum albumin	—
Penicillin *G*	—
Egg albumin	+++

P.C.A. titre of rabbit antibody was unchanged after heating at 56° C. for 4 hr.

Crossed antigenicity to various penicillin derivatives and degradation products were tested by the P.C.A. method. Penicillin *O* (allylmercaptomethyl-penicillin), penicillin *V* (phenoxymethyl-penicillin) and benzethicillin (dimethoxyphenyl-penicillin) gave the same positive results as penicillin *G*. Exceptionally, phenethicillin (DL-phenoxyethyl-penicillin) needed acidification to make combination with albumin in order to cause positive P.C.A. reaction.

Degradation products such as penicilloic acid, penicillic acid and penicillenic acid incubated with albumin gave no P.C.A. reaction against the antibody to penicillin *G*–albumin complex, while inhibition was observed to result from antiserum pre-incubated with these degradation products.

Mechanism of Antigenic Binding of Penicillin to Albumin

After incubation of penicillin *G* and albumin at 37° C. for 24 hr., the *p*H of the medium decreased from 6·2 to 4·6–4·8. By making the medium alkaline the P.C.A. titre was almost completely lost. On the contrary, by acidification the binding process was accelerated. The relation between decrease of *p*H and P.C.A. titre is shown in Table 2.

An incubated mixture of penicillin *G* and serum did not induce P.C.A. but after acidification and dialysis positive reaction was obtained. Tissue homogenate such as liver, spleen, lung and skin gave no positive result, with the exception of the exudates, which usually have acid reaction. From these observations it is obvious that penicillin may block alkaline

Table 2. Relation between Decrease of *p*H and Titre of P.C.A.

Antigens	*p*H before incubation	*p*H* after incubation	Titre of† P.C.A.
Penicillin *G* + Albumin	6·2	4·4	1,000
Penicillin *G* + Albumin (acidified)	4·6	4·6	1,000‡
Penicillin *G* + Albumin (alkalized)	7·2	5·8	1
Phenoxyethylpenicillin + Albumin	6·2	5·6	0
Phenoxyethylpenicillin + Albumin (acidified)	4·6	4·6	1,000
Penicillin *G* + Normal serum	7·2	7·0	0
Penicillin *G* + Normal serum (acidified)	5·0	4·4	100
Penicillin *G* + Dialysed serum	6·4	5·0	1,000
Penicillin *G* + Pleural exudate	6·2	5·2	100

* 24 hr. incubation at 37°C.
† maximum dilution of antiserum
‡ 30 min. incubation

radicals of the albumin molecules from the antigenic binding, whereas acidification accelerates this reaction. By means of penicillin *G* labelled with sulphur-35 the number of molecules of penicillin bound to one molecule of the albumin complex antigen was estimated as 44·5 (mean of 4 experiments). In autoradiographs of paper electrophoresis with serum or exudate, penicillin was found to be bound to albumin and not globulin.

A Peculiar Protein found in the Serum of Atopic Patients

The serum from patients with anaphylactoid reaction or angioneurotic œdema had strong capacity for antigenic binding without acidification. This binding occurred within 30 min. and was independent of the presence of any reagin to penicillin (Table 3).

Table 3. Capacity of Antigenic Binding of Serum from Normal Subjects and Patients

	Antigens	Duration of incubation	Terminal *p*H	Titre of P.C.A.
1.	Normal serum + Penicillin *G* (5 cases)	24 hr.	6·8–7·2	0
2.	No. 1 serum* + Penicillin *G*	24 hr.	7·0	1,000
	" + Penicillin *G*	30 min.	7·0	100
	" + Penicillin *V*	24 hr.	7·0	1,000
	" + Phenoxyethyl-penicillin	24 hr.	7·0	1,000
	" + Penicillioic acid	24 hr.	5·8	0
	Heated serum (56° C., 4 hr.) + Penicillin *G*	24 hr.	7·0	1,000
3.	No. 2 serum† + Penicillin *G*	24 hr.	6·8	1,000
4.	No. 3 serum‡ + Penicillin *G*	24 hr.	7·0	1,000

* Patient No. 1 with anaphylactoid reaction to penicillin, reagin to penicillin (+++).

† Patient No. 2 with anaphylactoid reaction to sulpha drug, reagin to penicillin (—).

‡ Patient No. 3 with angioneurotic œdema, reagin to penicillin (—).

From these results and observations we assumed the existence of a peculiar protein in such patients which has strong capacity of antigenic binding to some kind of haptene so that atopic patients acquire hypersensitivities very easily to many kinds of haptenes.

Physicochemical properties of this protein and implications respecting haptenes other than penicillin remain to be investigated.

[1] Welch, H., Lewis, C. N., Weinstein, H. I., and Boeckman, B. B., *Antibiotics Annual*, 296, 1957-58 (Medical Encyclopedia, Inc., New York, 1958).
[2] Fisher, J. P., and Cooke, R. A., *J. Allergy*, **28**, 150 (1957).
[3] Ovary, Z., *Progress in Allergy*, **5**, 459 (S. Karger, Basel, New York, 1958).

INHERITED CASEIN VARIANTS IN COW'S MILK

By Dr. R. ASCHAFFENBURG
Chemistry Department, National Institute for Research in Dairying, Shinfield, Berkshire

PAPER electrophoresis has been used successfully to demonstrate the occurrence of genetically distinct types of β-lactoglobulin[1,2] and α-lactalbumin[3] in cow's milk. With the aid of the same method, evidence has now been obtained for the existence of gene-controlled variants of the β- and γ-components of the principal milk protein, casein.

Paper electrophoresis of casein in conventional buffer systems presents difficulties which stem from the anomalous behaviour of α-casein. Zhdanova and Vlodavets[4] and Libbey and Ashworth[5] have shown that this fault can be eliminated by adding urea to the buffer, but they did not obtain a clean separation of β- from α-casein. Such a separation has now been achieved by working with a buffer system made up of constituents with which casein is associated under natural conditions, namely, citrate and phosphate. Briefly, the technique consists of electrophoresis at 4° C. in a buffer of *p*H 7·15, made up of 0·913 gm. citric acid monohydrate, 3·56 gm. disodium hydrogen phosphate and 360 gm. urea a litre. Using the horizontal type of apparatus described by Kohn[6], a constant 180 V. is applied for 5 hr. to two sheets of Whatman 3 *MM* paper (10 × 20 cm.), each carrying three or four samples deposited across the centre of the sheets. Samples are prepared by isoelectric precipitation of the casein from 10 ml. of skim milk, addition of 2 gm. urea to the centrifuged and washed casein, and dispersion with water to a final volume of about 5 ml. of a slightly opaque solution.

As shown in Fig. 1, the procedure resolves the protein into two α-casein bands, the slower-moving one probably representing the κ-casein of Waugh and von Hippel[7], a single β-band, and two γ-bands which move towards the cathode. Contrary to the other components, the γ-caseins—very clearly shown in the chosen examples—vary markedly in strength from milk to milk, as will be discussed later. Otherwise rather uniform results were obtained in comparing the casein patterns from the milk of individual cows. A departure from the norm emerged during the examination of a series of samples from one-egg twins of different breeds, kept at the Institute: milk from each of a pair of cross-bred Jersey–Guernsey cattle (O37/38) was found to contain two β-casein

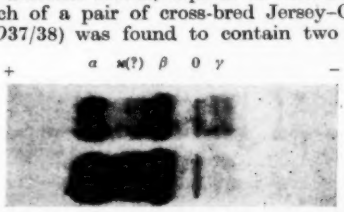


Fig. 1. Separation of casein by paper electrophoresis. 0 = origin (photographed in transmitted light)

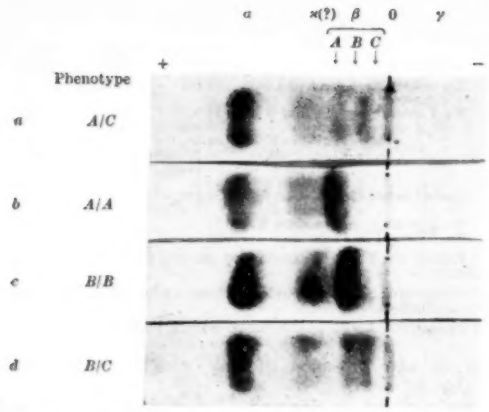


Fig. 2. β-Casein variants. 0 = origin (photographed in reflected light)

components (*B* and *C*; Fig. 2*d*), migrating more slowly than the usual single component (*A*; Fig. 2*b*). The fact that the 'anomaly' occurred with both twins, suggested a genetical basis, and led to the collection of samples at the farm of origin with the surprising result that, of 21 cows examined, only 3 produced β-casein *A*. Three additional variants, *A* and *C* (Fig. 2*a*), *A* and *B* (not shown), and *B* only (Fig. 2*c*) were found among the remaining samples. The older animals at this farm were Guernseys, the younger ones *F*1-crosses with Jersey sires. The male line was traced back to a small pedigree Jersey herd with ten cows in milk: four of these produced β-casein *A*, the others *B* or *A* and *B* combined.

Examination of the available family data showed the results to be in accord with the concept of the existence of three alleles of a gene (locus symbol β-*Cn*), giving rise to six possible phenotypes of which all but one, *C*/*C*, have so far been observed. Some relevant relationships for the twins *O*37/38 are given in Fig. 3. They show that all three alleles are present in the Guernsey stock, and that the Jersey sire (III) as well as its sire (II) and grand sire (I) transmit the *B*-allele which, as mentioned here, is prevalent at the Jersey farm.

Though still in its early stages, a survey of milk samples from cows of other Channel Island herds has already shown that the observations connected with the twins *O*37/38 are not to be regarded as rare anomalies. In tests of samples from seven Guernsey herds, both the *B*- and *C*-alleles were found in one herd and the *B*-allele in another; and in one of three

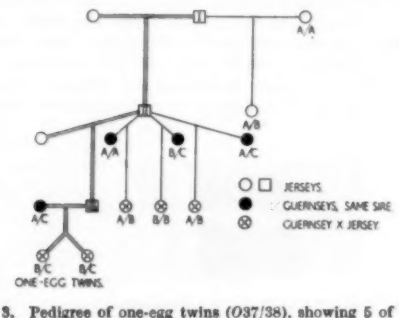


Fig. 3. Pedigree of one-egg twins (*O*37/38), showing 5 of the 6 possible phenotypes of β-casein

Jersey herds the *B*-allele occurred quite frequently. These herds are now under more detailed investigation, and further data on Jersey cows are being collected to ascertain whether the *C*-allele, so far only found in Guernsey milk, occurs in that of Jersey cows as well. Information is also required on the possible occurrence of the β-casein polymorphism in other breeds. So far only Friesian cows have been examined in sufficient number to suggest that the polymorphism might be restricted to Channel Island cattle. This would be highly interesting, since Bangham[8] has shown that hæmoglobin polymorphism among British cattle breeds is confined to Channel Island (and South Devon) animals. It is true that during tests on milk from more than a hundred Friesian cows, a few instances were observed in which the *B*-allele was present, but these were so isolated that they might well be merely the consequence of cross-breeding with Channel Island cattle.

As mentioned earlier, the two γ-casein bands, obtained from the milk of individual cows, were found to vary considerably in strength, as illustrated by Figs. 1 and 2. (The photographic reproduction conceals the fact that weak bands were present in all the examples shown in Fig. 2). In some samples, the bands appeared to be completely absent. The statement that this variability has a genetical basis is founded largely on the observation that, in comparisons of the strips prepared from the milk of eighteen pairs of one-egg twins, γ-caseins of identical appearance and strength were obtained within each single pair, while the variability between the pairs covered the whole range. These findings suggest the existence of a metabolic polymorphism of the kind described by Allison[9] as "that in which one genotype has a particular substance in relatively large amounts while other genotypes have smaller amounts or lack it altogether". There has been no indication that this polymorphism differs from breed to breed. Interpretation of the results is far from easy, when the γ-casein bands are very weak, and detailed study of this polymorphism has been postponed pending the development of a more searching technique producing the weak γ-caseins in higher concentration. Such a development seems necessary, as the simple expedient of applying more than the usual amounts of total casein to the filter paper results in the formation of blurred, poorly resolved bands.

Casein is present in cow's milk in the form of a colloidal complex. It appears from the foregoing that several independent genes are involved in its elaboration by the mammary gland, and that the 'classical' sub-division of the complex into α-, β- and γ-caseins is justified and meaningful.

I am grateful to my colleagues of the Dairy Husbandry Department, Dr. F. H. Dodd and W. S. Mitchell, and to N. T. Culling and his staff at the Reading Regional Milk Records Office, Milk Marketing Board, for the collection of milk samples and information; and to Miss J. E. Cooper for skilful technical assistance.

[1] Aschaffenburg, R., and Drewry, J., *Nature*, **176**, 218 (1955).
[2] Aschaffenburg, R., and Drewry, J., *Nature*, **180**, 376 (1957).
[3] Blumberg, B. S., and Tombs, M. P., *Nature*, **181**, 683 (1958).
[4] Zhdanova, E. A., and Vlodavets, I. N., *Biokhimia*, **24**, 371 (1959).
[5] Libbey, L. M., and Ashworth, U. S., *J. Dairy Sci.*, **44**, 1016 (1961).
[6] Kohn, J., *Clin. Chim. Acta*, **2**, 297 (1957).
[7] Waugh, D. F., and von Hippel, P. H., *J. Amer. Chem. Soc.*, **78**, 4576 (1956).
[8] Bangham, A. D., *Nature*, **179**, 467 (1957).
[9] Allison, A. C., *Amer. Nat.*, **93**, 5 (1959).

COMPARISON OF TRANSDUCTION VERSUS TRANSFORMATION OF LINKED MARKERS IN *BACILLUS SUBTILIS*

By ERELA EPHRATI-ELIZUR and MAURICE S. FOX

The Rockefeller Institute, New York, 21

WITH *Bacillus subtilis*, it has been possible by transformation to demonstrate linkage between mutant sites (markers) responsible for tryptophan and histidine requirements[1,2] and between markers responsible for tryptophan and tyrosine requirements[3]. The degree of the linkage is determined by comparing the frequency with which bacteria which have been transformed to independence of one required metabolite have been simultaneously transformed to independence of the other[4]. Due to the low frequency of transformation in these experiments, cells are rarely transformed simultaneously for two unlinked markers. As a result of the observation of phage-mediated transfer of genetic material (transduction) in *B. subtilis* by Thorne[5], it has become possible to compare the degree of linkage between a given pair of genetic markers introduced either by deoxyribonucleate mediated transformation or by bacteriophage-mediated transduction.

The lysogenic *B. subtilis W23 L–9*, carrying a transducing phage, was kindly provided by Dr. C. B. Thorne. Transducing phage suspensions were prepared in broth after confluent lysis in soft agar plates seeded with both the phage and the sensitive wild-type bacterial strain *W23* (ref. 6). Shaking with chloroform followed by a low-speed centrifugation was sufficient to remove the bacteria from these suspensions. The phage was partially purified by centrifugation at 13,000 r.p.m. for 80 min. Sterile glycerol was added to the phage suspension to make 10 per cent by volume and 1 ml. samples were stored in a freezer cabinet at −20° C. The phage titre was determined by plating on tryptone agar plates supplemented with 0·5 per cent glucose and 0·1 per cent yeast extract, using the agar layer method[6]. The transduction procedure described by Thorne was modified in the following way. Mutant bacterial cultures were grown in tryptone broth supplemented with 0·5 per cent sodium chloride and 0·5 per cent glucose, for 5 hr. at 37° C., with aeration. To 1 ml. samples, sterile glycerol was added to make 10 per cent by volume, and the cultures were kept frozen at −20° C. until use. For transduction experiments, the cultures to be transduced were allowed to thaw in an ice bath and either diluted 1/10 into fresh medium and incubated for 5 hr. or centrifuged after being thawed, re-suspended in 1 ml. of fresh broth and incubated with aeration for 60 min. Following either of these treatments, 0·1 ml. of a phage suspension was added and the cultures were incubated with aeration for 45 additional minutes after which aliquots were spread on plates containing minimal medium[3]. Control platings of the phage suspensions showed no bacterial contamination; deoxyribonuclease treatment of these suspensions had no effect on transduction yields. In the transduction tubes, the bacterial densities varied from 2 to 5 × 10^8/ml. Phage densities were between 2 and 3 × 10^9/ml. All incubations were at 37° C. The procedure for transformation has been described elsewhere[2].

It can be seen in Tables 1 and 2 that the frequency with which the respective pairs of markers are transferred jointly is very nearly the same, when they are introduced either by transformation or transduction. This is true for both linkage groups that have been examined, tryptophan–histidine and tryptophan–tyrosine. In Table 3 a comparison has been made between frequencies of transformation and transduction of tryptophan, and a number of markers located in another histidine region showing no evidence of linkage to tryptophan. The four mutant histidine sites represented in the first four strains have been shown by transformation to be

Table 1. LINKAGE BETWEEN HISTIDINE AND TRYPTOPHAN MARKERS AND BETWEEN TYROSINE AND TRYPTOPHAN MARKERS BY TRANSDUCTION AND TRANSFORMATION

Method 1

Recipient bacteria donor		Colonies acquiring his⁺			Colonies acquiring tryp⁺		
Strain No. genotype		Total his⁺-tryp⁺ examined	found	(Per cent) doubles	Total his⁺-tryp⁺ examined	found	(Per cent) doubles
168-*SB*25 his$_2$-tryp	Phage (*W*)	326	238	73 ± 6	297	220	74 ± 5
	DNA (*W*)	357	226	63 ± 7	340	221	65 ± 5
168-31 his$_3$-tryp	Phage (*W*)	320	204	64 ± 8	330	235	71 ± 6
	DNA (*W*)	387	241	62 ± 7	395	248	63 ± 7
		Colonies acquiring tyr⁺			Colonies acquiring tryp⁺		
		Total tyr⁺-tryp⁺ examined	found	(Per cent) doubles	Total tyr⁺-tryp⁺ examined	found	(Per cent) doubles
168-*SB*29 tyr$_1$-tryp	Phage (*W*)	280	157	56 ± 7	354	215	60 ± 9
	DNA (*W*)	335	182	54 ± 7	320	179	56 ± 6
168-*SB*30 tyr$_2$-tryp	Phage (*W*)	325	178	55 ± 4	296	168	57 ± 7
	DNA (*W*)	290	146	50 ± 5	287	150	52 ± 4

The cultures treated with deoxyribonucleate (DNA) or phage were plated on minimal agar supplemented with either histidine (his) or tryptophan (tryp), in the case of the his-tryp mutants, and with tyrosine (tyr) or tryptophan in the case of tyr-tryp mutants. Colonies selected for independence of one metabolite were tested for independence of the other by streaking on unsupplemented minimal agar plates. *W*, wild-type bacterial strain *W*-23.

Table 2. LINKAGE BETWEEN HISTIDINE AND TRYPTOPHAN MARKERS AND BETWEEN TYROSINE AND TRYPTOPHAN MARKERS BY TRANSDUCTION AND TRANSFORMATION

Method 2

Recipient bacteria donor		his⁺ colonies	tryp⁺ colonies	his⁺-tryp⁺ colonies	(Per cent) doubles
168-*SB*25 his$_2$-tryp	Phage (*W*)	1,226	1,280	854	68
	DNA (*W*)	1,173	1,246	812	67
168-31 his$_3$-tryp	Phage (*W*)	696	730	507	71
	DNA (*W*)	1,120	1,208	782	67
		tyr⁺ colonies	tryp⁺ colonies	tyr⁺-tryp⁺ colonies	(Per cent) doubles
168-*SB*29 tyr$_1$-tryp	Phage (*W*)	499	506	327	65
	DNA (*W*)	838	855	476	57
168-*SB*30 tyr$_2$-tryp	Phage (*W*)	1,237	1,125	623	53
	DNA (*W*)	870	1,081	484	50

The treated cultures were plated simultaneously on unsupplemented and singly supplemented minimal agar plates. The colonies appearing on minimal represent the doubly transformed/transduced cells.

$$\frac{\text{No. of colonies independent of both required metabolites}}{\text{Average number of singly transformed/transduced colonies}} \times 100$$

Table 3. Transduction and Transformation of Unlinked Tryptophan⁻, Histidine⁻ Mutants

Recipient bacteria Strain No.	genotype	•Colonies transduced to: his⁺	tryp⁺	his⁺-tryp⁺	•Colonies transformed to: his⁺	tryp⁺	his⁺-tryp⁺
168–26	his_4-tryp	157	1,750	0	440	400	0
168–30	his_8-tryp	37	536	0	107	85	0
168–(*N*-3)	his_{16}-tryp	5	75	0	1,020	850	0
168–*SB*1	his_1-tryp	28	130	0	1,020	1,100	0
168–32	his_4(?)-tryp	0	220	0	0	150	0

• Number of transduced or transformed colonies on plates, bearing approx. 10^7 treated bacteria.

closely linked to each other[2]. The tryptophan mutation is identical with that of the four mutants in Tables 1 and 2. It can be seen that in transduction, the frequencies of the genetic transfer of the histidine[1,9–10] region are less than 10 per cent of that of the tryptophan marker, whereas by transformation these markers are transferred at about the same frequency as is the tryptophan marker. In one mutant strain, 168–32, requiring tryptophan and histidine, neither mutational site has been precisely located. This mutant can neither be transformed nor transduced to histidine independence.

The results presented show that the ratio of the frequency with which a pair of markers is introduced simultaneously to the frequency of introduction of a single marker is the same, within experimental error, whether these markers are introduced by transformation or transduction. A group of histidine markers, shown to be unlinked to tryptophan by transformation, is also found to be unlinked by transduction. The frequencies of transfer of these latter histidine markers in comparison with tryptophan were always markedly lower in transduction than in transformation experiments. Differences in frequency of transduction of different markers have been described in *S. typhimurium*[7].

Thus it has been demonstrated that, for several linked pairs of markers, the process of genetic incorporation as expressed in the degree of linkage, is remarkably similar whether the transfer is mediated by purified deoxyribonucleate or by transducing bacteriophage.

We wish to thank Dr. R. D. Hotchkiss for the interest he has shown in this investigation.

The investigation was supported by a grant from the Association for the Aid of Crippled Children and grant No. *G*/12894 from the National Science Foundation to Dr. R. D. Hotchkiss.

[1] Nester, E. W., and Lederberg, J., *Proc. U.S. Nat. Acad. Sci.*, **47**, 52 (1961).
[2] Ephrati-Elizur, E., Srinivasan, P. R., and Zamenhof, S., *Proc. U.S. Nat. Acad. Sci.*, **47**, 56 (1961).
[3] Nester, E. W., *Bact. Proc.*, **61**, 90 (1961).
[4] Hotchkiss, R. D., and Marmur, J., *Proc. U.S. Nat. Acad. Sci.*, **40**, 55 (1954).
[5] Thorne, C. B., *Fed. Proc.*, **20**, 254 (1961).
[6] Adams, M. H., *Bacteriophages* (Interscience Publishers, 1959).
[7] Zinder, N. D., *J. Cell. Comp. Physiol.*, **45**, Supp. 2, 23 (1955).

EXTRACELLULAR ESTERASE OF GROUP A STREPTOCOCCI

Esterase in Extracellular Concentrates of Group A Streptococci and the Homologous Antibody

A CARBOXYLIC acid esterase sensitive to diisopropylfluoridophosphate (DFP) has been found in extracellular concentrates of group *A* streptococci. The existence in various sera of antibodies against this esterase is described. These antibodies precipitate the streptococcal esterase but do not block its activity, a situation identical to that found by Uriel[1] with the esterases of human serum and their corresponding horse antibodies.

The enzyme was demonstrated by the following method: Electrophoresis of ten-fold to a thousand-fold concentrates of the extracellular products of group *A* streptococci was made from holes or slots in agar gel for 75 min. in 10 × 4 cm. slides by the Grabar and Williams technique[2] or for 45 min. by the microscope-slide modification of Scheidegger[2]. Following electrophoresis or immunoelectrophoresis, the esterase activity was revealed by a technique described in ref 1. The principle of this method[3] involves the splitting of the substrate β-naphthyl acetate and coupling with the azo dye, diazo blue *B*, the esterase appearing as a red-blue area (Fig. 1). So far, esterase activity has been demonstrated in extracellular concentrates of six strains of group *A* streptococci (Table 1). The concentrated uninoculated broths gave negative results.

The esterase activity does not appear to be due to the following known streptococcal products; streptolysins *O* and *S*, streptokinase, proteinase, deoxyribonuclease, diphosphopyridine nucleotidase (DPNase), and erythrogenic toxins *A* and *B*. Of these the most interesting is proteinase, because it is known that some proteolytic enzymes like trypsin have esterase activity.

The electrophoretic mobility of the esterase was similar to that of streptolysin *O* (ref. 4). However, the two activities can be differentiated as will be shown here. The two products may share another property, as shown in Table 1. Concentrates of lot 32369–26, which had been prepared by filtration of the culture through a Berkefeld filter, showed no esterase activity; a similar adsorption had occurred with the streptolysin *O* of this lot[5].

Investigations of the effects of inhibitors were carried out by the method in ref. 1. DFP at 10^{-4} *M* inhibited the esterase activity, while 10^{-4} *M* *p*-chloromercuribenzoate was without effect. The opposite results were obtained with streptolysin *O* (ref. 4).

Table 1. Esterase Activity of Various Streptococcal Preparations

No.	Strain and lot No.*	Supernatant (*S*) or filtrate (*F*) and degree of concentration	Esterase activity	Serological type of strain	Main feature of strain
1*a*	32369-22	*S*-1000 ×	+	18	Production of erythrogenic toxin *B*
b	32369-26	*F*-1000 ×	−		
2*a*	Cook *B*-25	*S*-1000 ×	+	Unknown	Production of erythrogenic toxin *B*
b	Cook *B*-27	*S*-1000 ×	+		
3	*NY*5 (*E*14)-2	*S*-1000 ×	+	12	Production of erythrogenic toxin *A*
4*a*	*C*203*S*-9†	*S*	−	3	DPNase +
b	,,	*S*-30 ×	+		
5*a*	Wilson-1†	*S*	−		
b	,,	*S*-30 ×	+	1	DPNase −
6*a*	*S*-84-1	*S* (‡)	−	3	
b	,,	*S*-10 ×	+		Production of high yield of streptolysin *O*
c	,,	*S*-30 ×	+		

• Methods of mass culture were used as in ref. 5.
† Given by Dr. A. Bernheimer.
‡ Given by Dr. R. Wahl.

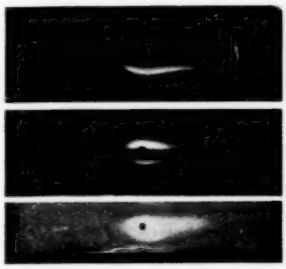


Fig. 1. *a*, Electrophoresis of extracellular concentrate Wilson-1 ; *b–e*, immunoelectrophoresis of extracellular concentrate Wilson-1; *b*, normal horse serum; *c*, normal human serum No. 1; *d*, normal human serum No. 2, 1 : 100; *e*, normal human serum, No. 2, 1 : 1,000

In immunoelectrophoresis, the various sera placed in the trenches included six normal adult sera, a 10 per cent human γ-globulin (Centre de Transfusion, Réanimation de l'Armée), one standard horse anti-streptolysin *O* serum (1,500 units/ml. Institut Pasteur) and scarlet fever antitoxin (horse), Lederle lot 1570–473 (ref. 5). All these sera gave arcs of enzymic activity as shown in Fig. 1, due to precipitation of antigen and antibody. The arcs of precipitate before staining for esterase were not visible to the naked eye nor could they be observed after staining with azocarmine[6]. A rabbit antiserum containing 1,500 units of antistreptolysin *O* and a rabbit anti-streptococcal serum (type 1, precipitating) made with killed bacteria gave negative results in immuno-electrophoresis.

That normal human serums can react with extracellular streptococcal antigens has been shown by Halbert *et al.* and confirmed by others (ref. 7), and the present findings confirm another antigen–antibody system.

Dr. Tom Webb and Efstratios Avrameas of this Service made useful suggestions in the course of the work. Sera were given by Drs. R. Wahl or A. Eyquem of the Institut Pasteur. Some streptococcal fractions were given by Dr. A. Bernheimer of New York University and Dr. S. P. Halbert of Columbia University.

Aaron H. Stock
José Uriel
Pierre Grabar

Electrophoretic Mobility and Detection of Hæmolytic Activity of Streptolysins O and S in Agar-Gel

While investigating the extracellular esterase of group *A* streptococci, it became necessary to have a direct method for the demonstration of the electrophoretic mobility of streptolysins *O* and *S*. A slight modification of the procedure of Uriel[8] for the direct detection of proteolytic enzymes after electrophoresis in agar-gel proved successful and is as follows: The streptolysin preparations were placed in slots cut in agar on slides. The streptococcal concentrates containing streptolysin *O* were either a crude thousand-fold concentrate of supernatants of broth cultures grown in dialysate broth[5] or a thirty-fold concentrate (as in ref. 5) of an Institut Pasteur streptolysin from cultures of strain *S* 84 of Kalbak grown in infusion broth (given by Dr. R. Wahl, Institut Pasteur, Paris). Both streptolysin *O* preparations contained sufficient lysin in the reduced form, so that addition of cysteine was not necessary for activity, which was fortunate, since this reagent nullified the effect of one of the inhibitors (*p*-chloromercuribenzoate) used in the investigation. That streptolysin *O* collected after 12–24 hr. can exist in the reduced form without reduction was shown in the pioneering studies of Neil and Mallory[9]. The nucleic acid induced streptolysin *S* was given by Dr. A. Bernheimer of New York University. Electrophoresis in agar-gel was carried out for 45 min. by the procedure in ref. 2 in a modified microscope-slide method of Scheidegger (see ref. 2). Following electrophoresis, the slides were immersed in phosphate-buffered saline *p*H 6·5 (ref. 10) for 15 min. to convert the gels to isotonicity. The surface of the slides was then dried lightly with filter paper, and overlayed by means of a pipette with 2 ml. of a 2 per cent washed human red blood cell suspension in melted 1·5 per cent agar in phosphate-buffered saline at 45–50° C. When the overlay had hardened, the slides were placed in Petri dishes and incubated at 37° C. In about 30 min. the hæmolysis became visible, but the reaction was allowed to proceed for a total of 60 min. As shown in Fig. 2, streptolysin *O* migrated very slowly while streptolysin *S* exhibited rapid mobility, easily differentiating the two lysins.

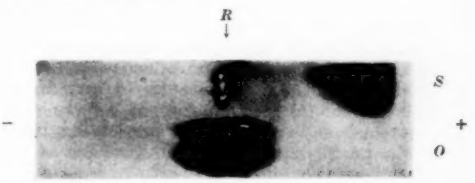


Fig. 2. Electrophoresis of streptolysins *O* and *S*. *R*, reservoir; top, streptolysin *S*; bottom, streptolysin *O*. The dark areas are the hæmolysed zones. There is an artefact at the reservoir of streptolysin *S* due to overlay of agar over the reservoir

The mobility of streptolysin *O* observed by the direct method apparently differs from the mobility described by Hanson and Holm[11] with the use of the 'basin overlay' and immunoelectrophoretic techniques and markedly differs with the mobility found by Halbert and Keatinge[7] with an immunoelectrophoretic method. By including in the overlay of red blood cells sufficient antistreptolysin *O* (horse, Institut Pasteur), it was demonstrated that streptolysin *O* activity could be inhibited.

Some investigations of chemical inhibitors were carried out using the technique elaborated by Uriel[1] for the study of esterases in human serum. Following the electrophoresis of streptolysin *O* or *S*, the slides were immersed in either 10^{-4} *M* di-isopropyl-fluorophosphate (DFP) or 10^{-4} *M* *p*-chloromercuri-benzoate in buffered saline, and overlays of red blood cells in agar were made, but containing 10^{-4} *M* concentration of the inhibitors. It was found that streptolysin *O* was inhibited by *p*-chloromercuri-benzoate in accordance with the findings by Smythe and Harris[12] of the effect of mercury salts on this lysin; it was not inhibited by DFP. The two inhibitors thereby differentiate streptolysin *O* from streptococcal esterase. Streptolysin *S* was not inhibited by either *p*-chloromercuribenzoate or DFP. To our knowledge these findings have not been reported before.

It is planned for the future to carry out electrophoretic work on other bacterial hæmolysins, especi-

ally tetanolysin, pneumolysin and the *O* toxin of *Cl. welchii*, all of which were shown by Todd[12] to have an immunological relationship to streptolysin *O*.

This work was aided by a grant from the National Institutes of Health, Bethesda.

A. H. Stock*
José Uriel

Service de Chimie Microbienne,
Institut Pasteur,
Paris.

* Present address: Department of Microbiology, School of Medicine, University of Pittsburgh.

[1] Uriel, J., *Ann. Inst. Pasteur*, **101**, 104 (1961).
[2] Micheli, A., and Grabar, P., *Ann. Inst. Pasteur*, **100**, 569 (1961).
[3] Nachlas, M. M., and Seligmann, A. M., *J. Nat. Cancer Inst.*, **9**, 415 (1949).
[4] Stock, A. H., and Uriel, J., see following communication.
[5] Stock, A. H., and Lynn, R. J., *J. Immunol.*, **86**, 561 (1961).
[6] Uriel, J., in Grabar, P., and Burtin, P., *L'analyse immunoélectrophorétique ; ses applications aux milieux biologiques humains* (Masson et Cie, Paris, 1960).
[7] Halbert, S. P., and Keatinge, S. L., *J. Exp. Med.*, **113**, 1013 (1961).
[8] Uriel, J., *Nature*, **188**, 853 (1960).
[9] Neil, J. M., and Mallory, T. B., *J. Exp. Med.*, **44**, 241 (1926).
[10] Herbert, D., *Biochem. J.*, **35**, 1116 (1941).
[11] Hanson, L. A., and Holm, S. E., *Acta Path. Microbiol.*, **52**, 59 (1961).
[12] Smythe, C. V., and Harris, T. N., *J. Immunol.*, **38**, 283 (1940).
[13] Todd, E. W., *Brit. J. Exp. Path.*, **22**, 172 (1941).

A CELL FOR RAPIDLY MEASURING RESTRICTION TO DIFFUSION OF MACROMOLECULES THROUGH AGAR GELS OF VARIOUS PORE SIZES

By G. K. ACKERS and R. L. STEERE

Plant Virology Laboratory, Crops Research Division, U.S. Department of Agriculture, Beltsville, Maryland

RESTRICTED diffusion of small proteins through stretched dialysis tubing has been investigated by Craig *et al.*[1]. This and other work indicated that restricted diffusion may be an effective tool for (*a*) size estimation of macromolecules in undefined or impure systems, (*b*) fractionation of components in such systems, and (*c*) tests of particle-size homogeneity in purified preparations. In view of these possibilities we thought it advisable to extend measurements into the range of pore sizes which would be effective in restricting diffusion of virus particles.

Rapid and reproducible results are obtained by using diffusion cells of the construction shown in Fig. 1. The inner chamber is made of glass. A thin piece of 'Monofilament Saran' screening (*MS*-904, 51 mesh, 0·008 in. yarn diam., National Filter Media Corp.) is stretched across its bottom and cemented securely around the edge. (The materials mentioned were purchased from the companies designated. These sources are presented only for specific identification of the materials used. No discrimination is intended and no guarantee of reliability implied.) The mesh on the bottom of this inner chamber is then immersed to a depth of approximately 1 mm. in a warm agar sol of accurately known concentration. Sol is drawn swiftly by surface tension into the interstices of the 'Saran' mesh. The chamber is withdrawn from the agar and excess sol is gently scraped from the bottom surface of the 'Saran' and from the outer rim of the chamber. A thin film of agar (about 0·03 cm. thick) is left in the mesh across the lower surface of the chamber. This film is allowed to gel at room temperature and forms a continuous, sturdily supported membrane. The cell is assembled with a rubber sleeve, a flat cover, a screw clamp, and an outer chamber of the dimensions shown. The inner chamber is filled with buffer solution and closed securely with the screw clamp. The membrane assembly is placed in contact with buffer in the outer chamber and allowed to 'mature' for 48 hr. in a cold constant-temperature chamber. The buffer in the inner chamber is then replaced by a buffered solution of the molecular species the diffusion of which is to be measured. That in the outer chamber is replaced by fresh buffer at successive timed intervals and concentrations of the diffusates are assayed.

Integration of Fick's first law for the boundary conditions of this system leads to equation (1) (ref. 2)

$$\frac{DA}{l} = -\frac{V_i V_o}{t(V_i + V_o)} \ln \frac{(C_i - C_o)f}{(C_i - C_o)i} \qquad (1)$$

D is the diffusion coefficient. *l* and *A* are the effective length and total cross-sectional area of pores in the

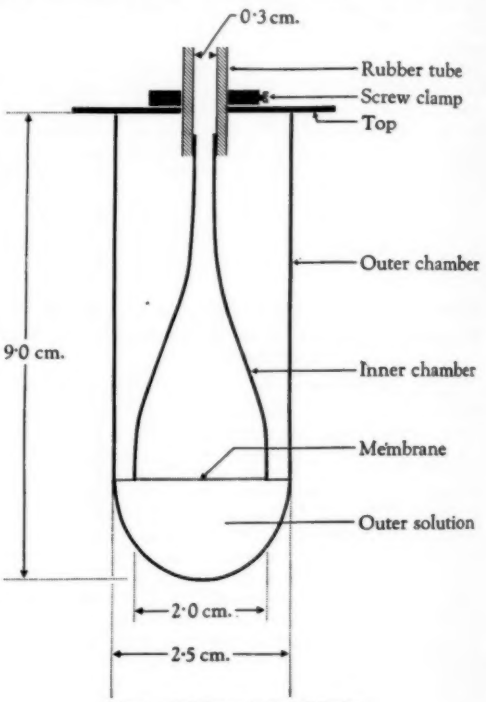


Fig. 1. Cell for restricted diffusion

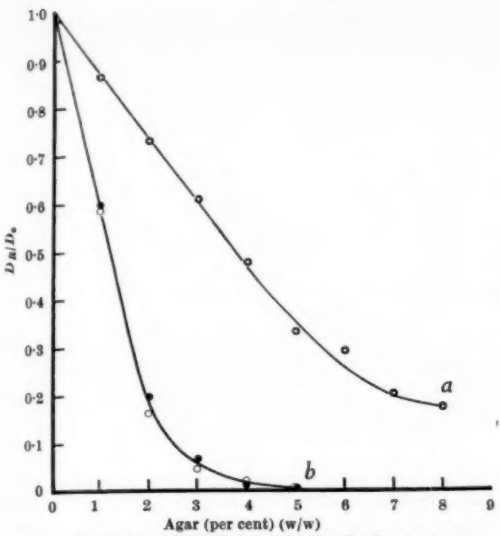


Fig. 2. Comparison of diffusional restriction for human hæmoglobin and southern bean mosaic virus particles. The restricted diffusion coefficients are plotted as percentages of the extrapolated diffusion coefficients against gel concentration. *a*, Human hæmoglobin; *b*, southern bean mosaic virus; ●, assay by ultra-violet absorbance; ○, assay by infectivity

membrane. V_i and V_o are the respective volumes of the inner and outer solutions the concentrations of which are C_i and C_o; i and f refer to initial and final conditions; t is the duration of the experiment. In practice the quantity A/l is evaluated for each membrane by diffusion of a small molecular species the diffusion coefficient of which is known.

Representative data (averages of 4 determinations) for restricted diffusion of purified southern bean mosaic virus (0·27 mgm./ml.) and 1 per cent human hæmoglobin (2 × cryst., 9019, Nutritional Biochemicals Corp., Cleveland, Ohio) both in 0·0667 *M* phosphate buffer (*p*H 6·7) through a series of 'Ionagar No. 2' (Oxoid) membranes of different gel concentrations are shown in Fig. 2. The agar was made up at each concentration (w/w) in distilled water and autoclaved 15 min. at 15 lb./in.² pressure. The cells were assembled and calibrated with 0·1 *M* sodium chloride (using $D_{20,w} = 1{\cdot}36 \times 10^{-5}$ cm.²/sec.) (ref. 3). Behaviour of all cells conformed closely to equation (1). This indicates that transport through the gel under the experimental conditions used is at least predominantly diffusional.

Diffusion was carried out in a constant-temperature chamber at 8° ± 0·5° C.. Suspensions were assayed by ultra-violet absorbance at 2600 Å. (for southern bean mosaic virus) and 2800 Å. (for hæmoglobin). The southern bean mosaic virus diffusates were assayed also by infectivity tests. Results of these measurements agree closely with the absorbance values as shown in Fig. 2.

Values for restricted diffusion coefficients were calculated by using equation (1) for the two species of particles at each gel concentration. When these coefficients (corrected to water at 20° C.) were plotted against agar concentration and the curves extrapolated to zero gel concentration, the values obtained were $1{\cdot}23 \times 10^{-7}$ cm.²/sec. for southern bean mosaic virus and $6{\cdot}6 \times 10^{-7}$ cm.²/sec. for hæmoglobin. These values are in good agreement with the reported values of $1{\cdot}39 \times 10^{-7}$ cm.²/sec. and $6{\cdot}9 \times 10^{-7}$ cm.²/sec. (ref. 4). Fig. 2 shows the comparative diffusional restriction encountered by particles of these two types as the gel pore size is decreased with increasing agar concentration. In this graph, ratios of D_R (the restricted diffusion coefficient) to D_o (the extrapolated coefficient) for the two particle-species at each gel concentration are plotted against the gel concentrations. It is clearly seen that the larger southern bean mosaic virus particles (28·0 mμ diam.[5]) were restricted to a much greater extent in their migration through the gel matrix than were the smaller (6·16 mμ diam.[6]) hæmoglobin particles.

Results of this investigation suggest that restricted diffusion by use of agar-gel membranes should be a particularly useful tool for the study of viruses. For example, although the hæmoglobin molecule diffuses only 4·5 times as fast as the southern bean mosaic virus particle under free conditions with only hydrodynamic restriction, it was found to diffuse 63 times as fast through a 4 per cent agar-gel membrane. With such separation between virus particles and other macromolecular components of tissue extracts, it should be possible to effect a high degree of purification by using a limited number of countercurrent steps under properly selected restrictional conditions.

We wish to express our appreciation to James M. Parsons for help in the initial stages of this work.

A part of this work was carried out in the laboratories of the Harding College Chemistry Department, Searcy, Arkansas, under the support of a Brown Hazen grant obtained through Research Corporation, New York.

[1] Craig, L. C., Konigsberg, Wm., Stracher, A., and King, T. P., *Symp. on Protein Structure*, edit. by Neuberger, A., 104 (John Wiley and Sons, New York, 1958).
[2] Gordon, A. R., *Ann. N.Y. Acad. Sci.*, **46**, 285 (1945).
[3] Stokes, R. H., *J. Amer. Chem. Soc.*, **72**, 2243 (1950).
[4] Edsall, J. T., in *The Proteins*, edit. by Neurath, H., and Bailey, K., **1**, 634 (Academic Press, New York, 1953).
[5] Kaesberg, P., *Proc. First Nat. Biophys. Conf.*, 244 (Yale Univ. Press, New Haven, Conn., 1959).
[6] Pappenheimer, J. R., Renkin, E. M., and Borrero, L. M., *Amer. J. Physiol.*, **167**, 13 (1951).

AUTORADIOGRAPHY USING MELTED NUCLEAR EMULSION

By Dr. A. C. KULANGARA

Department of Embryology, Carnegie Institution of Washington, Baltimore 10, Maryland

AUTORADIOGRAPHY of tissue sections using melted emulsion may be carried out in two ways: the sections may be stained first, coated with nitrocellulose and emulsion and then processed, or the sections may be stained after photographic processing. During an investigation of rabbit uterus (labelled with carbon-14) by the first method, it was found that sections became de-stained in spite of considerable overstaining, and black deposits, ranging from a few silver grains to large aggregates, appeared

Table 1

Slide No.		Mean No. of grains ± stand. error in 1,380 μ^2		Mean No. of aggregates ± stand. error in 5,520 μ^2	Mean No. of grains ± stand. error over one nucleus
		Background	Over tissue		
1	Unstained sections covered with thin nitrocellulose coat	8·4 ± 0·59	16·0 ± 1·95	15·4 ± 2·05	
2	Unstained sections embedded in a thick nitrocellulose coat	6·0 ± 0·32	7·7 ± 0·76	none	
3	Sections stained in hæmatoxylin and covered with thin nitrocellulose coat	7·4 ± 0·71	18·1 ± 1·1	63·4 ± 5·71	15·75 ± 1·92
4	Sections stained in eosin and covered with thin nitrocellulose coat	7·1 ± 0·61	10·8 ± 0·76	22·2 ± 1·8	several

All sections were of non-radioactive rabbit uterus.
Grains were counted under oil immersion, in 1,380 μ^2 at a time, at least 10 such areas being counted for a sample. Aggregates were counted in 5,520 μ^2 at a time, four such areas being counted for a sample.

on slides. Discrete grains, indistinguishable from those caused by ionizing events, occurred in greater density near the aggregates.

The formation of aggregates has been reported by others[1,2], but discrete grains which may occur with or without aggregates do not seem to have received as much attention. Stains and other solutions have been shown to remove radioactivity from tissue[3] and silver grains from images[4]. Several substances have been shown to cause chemical fogging[5], but a protective film between tissue and emulsion is considered to prevent such artefacts. The observations reported here indicate that stains may be involved in the formation of artefacts when a thin nitrocellulose film is used and that artefacts as well as bleaching of stains may be prevented by embedding sections in a hydrophobic layer.

Strain-induced artefacts. Slides with 8μ thick sections of non-radioactive rabbit uterus were deparaffinized and covered with a thin or thick coat of nitrocellulose. Kodak *NTB*3 emulsion was applied over these unstained sections, the slides sealed in light-tight boxes and processed after 24 hr. in the refrigerator (Fig. 1 ; Table 1, slides 1 and 2). Slide 1 showed several aggregates and the grain count over tissue was twice the background count ($P < 0{\cdot}001$), whereas slide 2 showed no aggregates and the grain count over tissue was not significantly different from background. Among slides with a thin nitrocellulose coat, appreciably fewer aggregates were found on those that were dried flat after dipping in nitrocellulose than on slides which were dried vertically.

The thin coat was applied by two dips in 1 per cent nitrocellulose, the slide being dried for 15 min. after the first dip and overnight after the second. The film which resulted was about 0·3μ thick and the emulsion formed a layer varying in thickness from 3 to 10μ. The thick coat was obtained by pipetting 1·5 ml. of 2 per cent nitrocellulose on the slide and drying in a slow stream of air for about 18 hr. The sections became embedded in a 9μ thick layer of nitrocellulose and the emulsion applied on its even surface formed a uniform layer about 3μ in thickness.

It has been suggested[2,4] that emulsion layers of uneven thickness may become reduced due to physical strains during drying or processing. Evidence confirming this view was obtained here when clean slides, slides with coverslips cemented on them and slides with depressions (5, 8, 10 and 15μ deep) ground on them were coated and processed as in the case of slide 1. Aggregates and grains were found at the edges of coverslips and depressions. It may be mentioned here that such strain-induced artefacts occur with 5μ or thicker sections ; they are less common or absent with thinner sections. Tissue sections with few spaces or small ones (less than about 30μ across), such as liver and spleen sections, also do not produce many artefacts.

Chemically induced artefacts. In order to determine whether stains could reduce the emulsion when they were separated from it by a thin nitrocellulose film, a drop each of Mayer's hemalum and 1 per cent eosin were dried on a slide, the slide was coated with

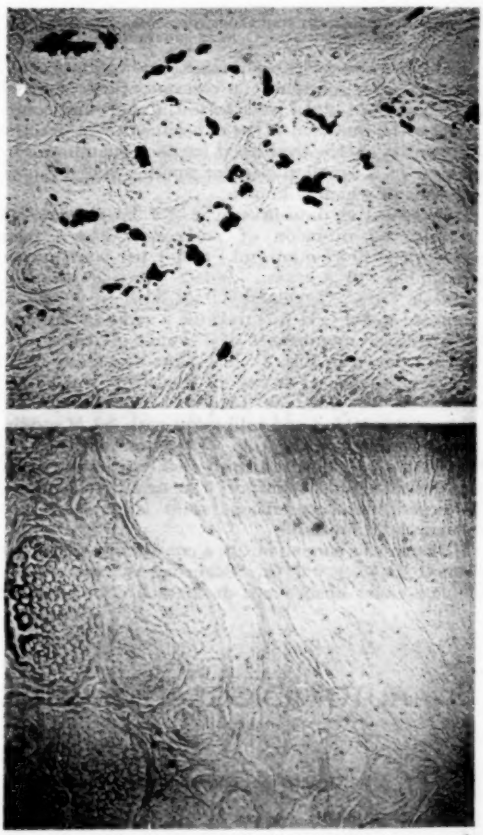


Fig. 1. Non-radioactive unstained section of rabbit uterus covered with a thin nitrocellulose coat (above) showing strain-induced aggregates and grains. Similar section embedded in a thick nitrocellulose coat (below) showing absence of aggregates ; grains over this section appear as out of focus images due to the thick nitrocellulose coat. (× 300)

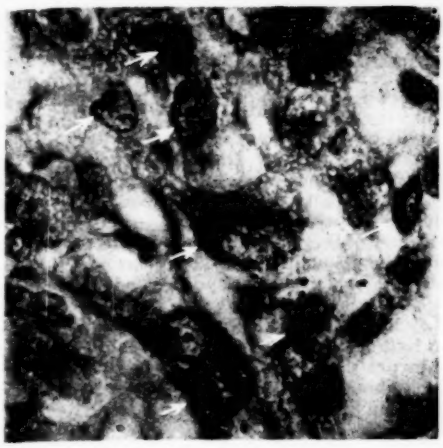


Fig. 2. Grain clumps (arrows) over non-radioactive nuclei (slide 4, Table 1). (× 1,000)

nitrocellulose and emulsion and processed. The grain counts over hemalum, eosin and background were $24 \pm 6{\cdot}1$, $12{\cdot}3 \pm 0{\cdot}86$ and $7{\cdot}2 \pm 0{\cdot}75$ respectively. Counts around the hemalum spot were 1·5–2 times background count, indicating some lateral diffusion of the reaction. When non-radioactive sections stained in hemalum were treated similarly, counts over tissue and background were 18·1 and 7·4 respectively (Table 1). The mean number of aggregates was 63·4 and the largest of them was 262μ. Corresponding results with sections stained in eosin were 10·8 and 7·1 grains over tissue and background, mean number of aggregates 22·2 and the largest of them measuring 210μ. All grain counts are significantly above background ($P < 0{\cdot}001$, except slide 4, where P is 0·03). The difference between aggregate counts over unstained sections and those stained with eosin is also significant ($P < 0{\cdot}02$). Aggregates on these slides were not confined to tissue spaces and edges as the strain-induced ones were. Slides on which sections were stained for 5, 10 and 15 min. in hemalum showed increasing grain count over tissue with longer staining time (5·9 : 13·3 : 14·9, $P < 0{\cdot}01$). Similar sections counterstained with eosin for 10 min. also showed an increase (8·7 : 10·8 : 15·3, $P < 0{\cdot}01$). The increase of grain count with longer staining time was abolished by a thick coat of nitrocellulose. These experiments show that stains, either directly or from tissues, may reduce the emulsion in spite of a thin nitrocellulose film.

Observations such as the effect of a thick nitrocellulose coat in reducing artefacts, and association of aggregates and grains with bleached areas of stained sections, indicated that the photographic developer and fixer (Kodak *D*19 and 'Acid Fixer'), diffused across the thin nitrocellulose film, reacted with the stains on sections and as a result stains were bleached and the emulsion reduced. Slides processed in a less alkaline *D*19 (made up with 3 gm. of sodium carbonate instead of 48 gm./l.) and 5 per cent sodium thiosulphate showed a grain count over tissue similar to background count and bleaching of stains was almost eliminated. Thus, these chemically induced artefacts seem to be the result of interaction between the stains and *D*19 and 'Acid Fixer'.

One other observation of grains appears worth recording. Slides 3 and 4 (Table 1) showed several nuclei over which grains occurred in clumps (Fig. 2). The mean of grain counts over 20 nuclei on slide 3 was 15·75. These artefacts are indistinguishable from autoradiograms of nuclei labelled with tritiated thymidine.

Protective coating. Systematic work[6–9] on protective films has tested a wide variety of substances. None of them seems to give complete protection and yet is satisfactory in other respects[10]. More recently 'Plexiglas' solution and acrylic membranes have been tried[11,12]. 'Harleco Synthetic Resin' (HSR), the routine mounting medium for histological preparations in this laboratory, was found to be a satisfactory protective coating. The sections may be coated after staining, dehydration and clearing in xylol in the following manner. Each slide is removed from xylol, the under surface and edges wiped dry and placed on a previously levelled metal platform. A volume of 2 per cent HSR in xylol that will produce a layer as thick as the sections is pipetted on the surface before the sections become dry. The linear relation between volume of 2 per cent HSR and final thickness of dry coat is obtained by prior calibration. When all slides are ready a 'Plexiglas' cover is placed over the platform and a stream of air drawn over the slides through an outlet on top of the cover using a squirrel cage blower. The slides are dried overnight and may then be coated with emulsion. The sections become embedded in the HSR layer, which presents a smooth and even surface to which emulsion will adhere.

Sections stained with toluidine blue, phosphotungstic hæmatoxylin and with two triple stains (Heidenhain's azan and by the method of Himes and Moriber[13]) were fully protected by HSR. The resin does not seem to reduce the emulsion by itself; after exposure of emulsion to the resin for 3 weeks, grain counts were not significantly different from background. HSR has excellent optical qualities[14] and is readily available (Hartman Leddon Co., Philadelphia). Since sections are embedded in HSR by this method, they never become dry during the entire process. Solvents like xylol cannot be used in permanent mounting of these autoradiograms; but the slides may be mounted in 4 per cent polyvinyl alcohol directly after photographic processing, thereby eliminating the dehydration and clearing used as a rule.

I am grateful to Dr. J. D. Ebert and Dr. B. G. Böving for their advice. I wish to thank Mr. W. H. Duncan for his suggestions concerning histological procedures.

[1] Doniach, I., and Pelc, S. R., *Brit. J. Radiol.*, **23**, 184 (1950).
[2] Joftes, D. L., *Lab. Invest.*, **8**, 131 (1959).
[3] Williams, A. L., *Nucleonics*, **8**, No. 6, 10 (1951).
[4] Boyd, G. A., *Autoradiography in Biology and Medicine* (Academic Press, New York, 1955).
[5] Yagoda, H., *Radioactive Measurements with Nuclear Emulsions* (John Wiley and Sons Inc., New York, 1949).
[6] Chapman-Andresen, C., *C. R. trav. Lab. Carlsberg, sér. chim.*, **28**, 529 (1953).
[7] Everett, N. B., and Simmons, B. S., *Anat. Rec.*, **117**, 25 (1953).
[8] Joftes, D. L., and Warren, S., *J. Biol. Photographic Assoc.*, **23**, 145 (1955).
[9] Gomberg, H. J., *Nucleonics*, **9**, No. 4, 28 (1951).
[10] Norris, W. P., and Woodruff, L. A., *Ann. Rev. Nucl. Sci.*, **5**, 297 (1955).
[11] Guidotti, G., and Setti, R. L., *Stain Tech.*, **31**, 57 (1956).
[12] Mérei, F. T., and Gallyas, F., *J. Histochem. Cytochem.*, **8**, 444 (1960).
[13] Himes, M., and Moriber, L., *Stain Tech.*, **31**, 67 (1955).
[14] Lillie, R. D., Zirkle, C., Dempsey, E. W., and Greco, J. P., *Stain Tech.*, **28**, 57 (1953).

LETTERS TO THE EDITORS

COSMOLOGY

Dirac's Cosmology and Mach's Principle

The dimensionless gravitational coupling constant

$$\frac{Gm_p^2}{\hbar c} \sim 5 \times 10^{-39} \quad (1)$$

with m_p the mass of some elementary particle, for definiteness taken as the proton, is such a small number that its significance has long been questioned. Thus Eddington[1] considered that all the dimensionless physical constants, including this one, could be evaluated as simple mathematical expressions. Dirac[2] considered that such an odd number must be related to other numbers of similar size, characterizing the structure of the universe. However, most physicists seem to believe that a dimensionless constant, such as (1), is provided by Nature, cannot be calculated, and is not in any way related to other numbers.

Dirac noted that most physical and astrophysical dimensionless constants are of the order of magnitude of integral powers (positive and negative) of the number 10^{40}, where such numbers as $m_p/m_e \sim 1{,}800$ and $\hbar c/e^2 \sim 137$ are said to be of the order of unity, the zero power of 10^{40}. He considered unlikely the accidental correspondence of the apparently unrelated, enormous numbers, and he suggested some unknown causal connexion.

One of these large numbers is:

$$T\frac{m_p c}{\hbar} \sim 10^{42} \quad (2)$$

with T the Hubble age of the universe. For an evolutionary universe, T varies with time. This suggested to Dirac that all the large numbers vary with time, with numbers of the order of $(10^{40})^n$ varying as T^n. Dirac[3] constructed a cosmology based on this idea, and Jordan[3] produced a proper relativistic theory of Dirac's cosmology.

The three principal large numbers are given by equations 1 and 2, and by the mass of the universe to its visible limits, expressed as:

$$\frac{M}{m_p} \sim 10^{80} = (10^{40})^2 \quad (3)$$

According to Dirac's hypothesis, these three numbers should vary as the -1, 1, and 2 power of the time respectively.

It should be noted that it is necessary to assume more than a connexion between the three numbers (1), (2), and (3) to justify Dirac's hypothesis. In addition, one must assume that the apparent interconnexion between the three numbers is independent of time. This assumption could be supported by a statistical argument. If the present value of T were to be considered conceptually as a random choice from a wide range of possible values of T, the present 'choice' would have had a small *a priori* probability, and an accidental correspondence of the type exhibited by the three numbers would have been unlikely. In view of the inexactness of the interrelation between the three numbers, a very wide range of possible values of T and a small *a priori* probability must be assumed if Dirac's hypothesis is to receive support from this type of argument.

It will be shown that, with the assumption of an evolutionary universe, T is not permitted to take one of an enormous range of values, but is somewhat limited by the biological requirements to be met during the epoch of man.

The first of these requirements is that the universe, hence galaxy, shall have aged sufficiently for there to exist elements other than hydrogen. It is well known that carbon is required to make physicists.

It is known that the galaxy was formed initially from hydrogen only. Hence, the minimum time for the start of the epoch of man is set by the age of the shortest-lived stars, for elements, other than hydrogen, are formed in the interior, and distributed at the death, of the star.

An upper limit for the epoch of man is set by the requirement that he has a hospitable home in the form of a planet circling a luminous star. This time is set by the maximum age of a star capable of producing energy by nuclear reactions. For stars less massive, gravitational contraction is halted by the onset of electron degeneracy pressure, before central temperatures rise high enough for the onset of nuclear reactions. The mass of the longest-lived stars can be computed assuming that electron degeneracy occurs at the nuclear reaction temperature. This gives for the lower bound on M_s, the mass of the star:

$$\frac{M_s}{m_p} \sim 10^{-3}\left(\frac{\hbar c}{Gm_p^2}\right)^{3/2} \quad (4)$$

where m_p is again the proton mass. According to the above definition of order of magnitude, 10^{-3} is to be regarded as equivalent to unity and this number is of the order of magnitude of $(10^{40})^{3/2}$.

The life-span of a star of this mass can be calculated, assuming that the emission and opacity are determined by free–free transitions (the bremsstrahlung radiation and absorption process). This lifetime is:

$$T_{\max.} \sim \left(\frac{m_p}{m}\right)^{5/2}\left(\frac{e^2}{\hbar c}\right)^3\left(\frac{Gm_p^2}{\hbar c}\right)^{-1}\frac{\hbar}{m_p c^2} \quad (5)$$

after dropping numerical factors, where m is the electron mass. Dropping the 'unity factors', this becomes:

$$\frac{m_p c^2}{\hbar} T_{\max.} \sim \left(\frac{Gm_p^2}{\hbar c}\right)^{-1} \quad (6)$$

in agreement with equations (1) and (2). In similar fashion, $T_{\min.}$ as determined by requirements for stellar stability, is of the same order of magnitude. Thus, contrary to our original supposition, T is not a 'random choice' from a wide range of possible choices, but is limited by the criteria for the existence of physicists.

Two problems remain. Why is the gravitational coupling constant so small? Why does the square root of the number given by equation (3) agree with the reciprocal of (1)? Both of these questions are answered by introducing Mach's Principle. According to the interpretation of Mach's Principle sometimes made[4–6], the gravitational coupling constant is not fixed but is determined by the mass distribution of the universe in such a way that:

$$\frac{GM}{c^3 T} \sim 1 \quad (7)$$

Combining equations (7) and (6) gives the expression obtained by combining equations (1) and (3).

The reason for the smallness of the gravitational coupling constant is the enormous amount of matter in the universe. This may not be a very satisfactory answer, and it cannot be completely satisfactory until the problem of mass creation is understood.

The statistical support for Dirac's cosmology is found to be missing. However, the existence of physicists now and the assumption of the validity of Mach's Principle are sufficient to demand that the order-of-magnitude relations between the three numbers, given by equations (1), (2) and (3), be satisfied.

R. H. Dicke

Palmer Physical Laboratory,
Princeton University,
New Jersey.

[1] Eddington, A. S., *Theory of Protons and Electrons* (Cambridge Univ. Press, 1936).
[2] Dirac, P. A. M., *Proc. Roy. Soc.*, **A, 165**, 199 (1938).
[3] Jordan, P., *Schwerkraft und Weltall* (Braunschweig, 1955).
[4] Sciama, D. W., *Mon. Not. Roy. Astro. Soc.*, **113**, 34 (1953).
[5] Dicke, R. H., *Amer. Scientist*, **47**, 25 (1959).
[6] Brans, C., and Dicke, R. H., *Phys. Rev.*, **124**, No. 3 (1961).

Dicke discusses the three cosmological numbers: (1) which determines the gravitational constant, (2) which determines the Hubble age of the universe, and (3) the number of particles in the universe. They are related in that: (1) is roughly the reciprocal of (2) and (3) is roughly the square of (2). I assumed that these relations correspond to something fundamental in Nature. With an evolutionary universe (2) varies with time, and then (1) and (3) would also have to vary with time.

Dicke considers that there is a fundamental relation between (1) and (3), following from Mach's Principle, but that (2) is independent, so that (1) and (3) may be constant while (2) varies with time. He then shows that (2) would have to have roughly its present value when habitable planets exist. On this assumption habitable planets could exist only for a limited period of time. With my assumption they could exist indefinitely in the future and life need never end.

There is no decisive argument for deciding between these assumptions. I prefer the one that allows the possibility of endless life. One may hope that some day the question will be decided by direct observation. One would have to measure (1) to an accuracy of 1 in 10^{10} and then repeat the measurement after a few years and see whether the result has altered.

P. A. M. Dirac

St. John's College,
Cambridge.

SPACE SCIENCE

Direct Measurement of Interplanetary Dust Particles in the Vicinity of Earth

Additional knowledge about interplanetary dust particles has been obtained from the direct measurements made with rockets, satellites, and space probes. The results obtained with the microphone type of dust particle sensor carried by the U.S. satellites, *Explorer I* (1958 Alpha) and *Vanguard III* (1959 Eta); the U.S. space probe, *Pioneer I*; and the Soviet vehicles, *Sputnik III* (1958 Delta 2), Space Rockets I and II, and the Interplanetary Station (1959 Theta), have already been reported. In addition, a definitive set of data has recently been obtained with the U.S. satellite, *Explorer VIII* (1960 Xi). A series of corrections has been completed which allows our results from a series of seven successful high-altitude rockets instrumented at Oklahoma State University to be used in quantitative discussions of interplanetary dust particles. We would like to present the preliminary results (including a new and very interesting curve for the mass distribution and spatial density) from an analysis of the available direct measurements of interplanetary dust particles.

The best calibrations of the microphone system that are at present possible are performed with particles ranging from large glass beads elastically impacting at low speeds to small iron spheres inelastically impacting at speeds up to approximately 10 km. sec.$^{-1}$. Such calibrations indicate that the microphone system is sensitive to a quantity that is closely related to the momentum of an impacting dust particle. An average velocity, relative to a satellite, of 30 km. sec.$^{-1}$ has been assigned to the dust particles in order that the results may be used in a determination of the mass distribution of the interplanetary dust particles.

The three datum points obtained with *Explorer VIII* are plotted (together with those from *Vanguard III*, *Explorer I* and the series of rockets) as the distribution curve shown in Fig. 1. The equation of a straight line that approximately fits the datum points is:

$$\log I = -17{\cdot}0 - 1{\cdot}70 \log m$$

where I is the influx-rate of dust particles and m is the particle mass holding approximately for m in the range 10^{-10} gm. $\lesssim m \lesssim 10^{-6}$ gm. The data from *Vanguard III*, *Explorer I* and the rockets fit remarkably well on the curve defined by the data from *Explorer VIII*. In fact, all other available direct measurements also can be made to look fairly reasonable when plotted as in Fig. 1 if proper allowances are made for the type of experiment, the confidence that can be placed in any particular measurement, the shielding effect of Earth, and the actual fluctuations in the spatial density that have been observed with *Explorer I*, *Vanguard III*, and *Explorer VIII*.

The curves obtained from an extrapolation of the tabulation by Watson[1] and from the extrapolated tabulation by Whipple[2] of results from meteor observations are shown as reference curves in Fig. 1. Both the extrapolated curves follow the constant mass per unit visual magnitude relationship common to discussions of results from meteor observations and roughly serve as the limits of uncertainty encountered in placing such results on a mass distribution curve. This uncertainty arises because of the uncertainty in relating particle mass to photographic, visual, or radar magnitude, and the uncertainty in assigning a mass density of meteoroidal particles.

Either of the extrapolated curves can be forced to pass through any one of the given direct measurements datum points by adjusting the mass to magnitude relationships and the mass density for meteoroidal particles. The three datum points obtained with *Explorer VIII* define a slope for a limited portion of the distribution curve in addition to defining the influx-rates for dust particles in three different ranges of particle mass, showing that the curve determined by direct measurements departs significantly from the extrapolated curves.

Caution must be exercised in extrapolating the new distribution curve out of the range of particle mass for which direct measurements have actually been

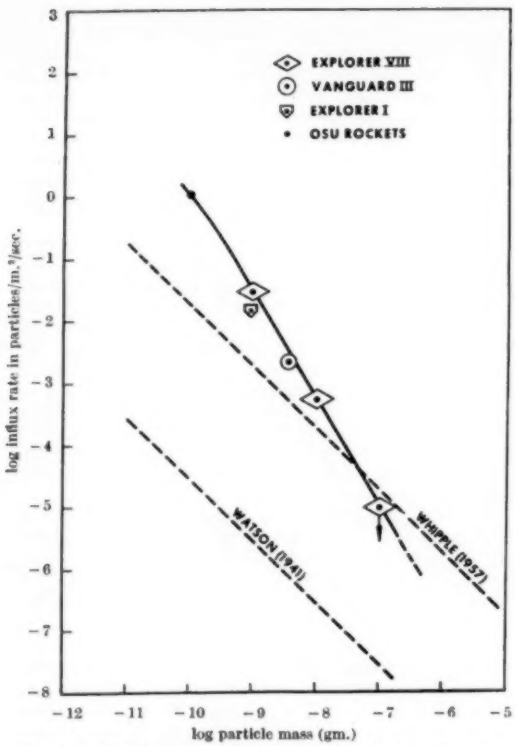


Fig. 1. Distribution curve for interplanetary dust particles in the vicinity of Earth

obtained. A considerable change in the shape of the distribution curve is bound to occur between the direct-measurements range of particle size and the range of particle size where the radiation pressure control becomes dominant unless there is a most prolific source of very small dust particles somewhere near or inside the orbit of Earth. The new distribution curve can be joined smoothly to the extrapolated curve labelled "Watson" at a size of particle smaller than the faintest radar meteors, but junction with the curve labelled "Whipple" is rather difficult.

An interesting consequence of the new distribution curve is that the daily influx-rate of interplanetary dust is approximately 10^4 tons a day on Earth, meaning that dust particles in the direct-measurements range of particle size dominate the accretion process. The meaning of the new distribution curve with regard to theories of the zodiacal light and observations of atmospheric aerosols bears further investigation.

The question whether the direct-measurements results show a dependence on altitude of the spatial density of dust particles has not been resolvable, on the basis of results obtained with the microphone type of impact detector, prior to obtaining the results of *Explorer VIII*. The reason for this difficulty was that the rocket measurements were of higher sensitivity and at lower altitudes while the reliable satellite measurements were generally of lower sensitivity and at higher altitudes. The satellite measurement at high sensitivity and at a large geocentric distance made by *Pioneer I* extended over only a short sampling interval and may not be of much use in establishing a model average distribution. Any one of several interpretations could be given to the available direct measurements, depending on the choice of a model distribution to which they were compared.

By assuming that the extrapolated curve labelled "Whipple" represented the true distribution, Whipple[3] has deduced that the direct measurements have confirmed the existence of a geocentric "dust belt". If the empirical curve defined by the data from *Explorer VIII* is used as a basis for an analysis of other direct measurements results, then no discernible dependence on altitude of the spatial density of dust particles exists. The conjectural geocentric concentration of dust particles under discussion here should not be confused with concentrations of dust-particles possibly suspended in the atmosphere of Earth at and slightly above the temperature inversion layer at about 80 km. altitude.

In conclusion, it should be emphasized that while the data at present available show no evidence for the existence of an appreciable geocentric concentration of dust particles, the data are really not of such a nature that they can be used in conclusively settling the question. If such a concentration does exist, then it certainly has not been convincingly measured by rockets and satellites. The direct measurements are quite useful in determining a new model of the average dust distribution, with the direct measurements results showing remarkable self-consistency when displayed as in Fig. 1.

C. W. McCracken
W. M. Alexander
M. Dubin

National Aeronautics and Space Administration,
Washington, D.C.

[1] Watson, F. G., *Between the Planets* (The Blakiston Company, Philadelphia, 1941).
[2] Whipple, F. L., *Proc. Eighth Intern. Astronaut. Congr.* (Springer-Verlag, Wien, 1958).
[3] Whipple, F. L., *Nature*, **189**, 127 (1961).

ASTRONOMY

Exact Determination of the Lunar Mass by means of Selenoid Satellites

As is well known, the astronomical determination of the mass of the Moon has so far been accomplished by methods based on the observation of the terrestrial nutation of the motion of the centre of the Earth around the centre of mass of the Earth–Moon system, and from the perturbation of the asteroid Eros.

The numerical value 0·01230 times the terrestrial mass is known to four significant figures, with an accuracy of about 4 parts in a thousand. It has been suggested[1,2] that this accuracy can be improved by observation of the motion of what we have called a 'selenoid satellite', that is, a satellite placed in the general vicinity of one of the sextile libration points of the Earth–Moon system. The reasons for believing that an improved accuracy would result by means of such a method are based on the fact that, in the first place, a prolonged time of observation would, in this case, be available; secondly, that the motion of such a body would be accurately predictable.

More specifically, the motion of a selenoid satellite could be qualitatively described[1,2] as a superposition of what we might call free and forced oscillations. The free oscillations would be those which the selenoid would perform if the Earth–Moon system were isolated in space. If the relative orbits of the Earth and the

Moon were exactly circular, the period of these oscillations would be respectively of 28·54 and 91·35 sidereal days. Owing to the effects of non-linear terms and eccentricity of the Earth–Moon orbits, additional periodic terms having as period all possible linear combinations of the above periods as well as of the period of the unperturbed Earth–Moon system will make their appearance. The forced oscillations will be due mainly to the action of the Sun, which will directly influence the orbiting satellites as well as the remaining components of the Earth–Moon system. The most conspicuous of the above oscillations will presumably be the free ones as well as those having periods equal respectively to the sum and difference of one year and the above free periods.

The proposed method of determination of the lunar mass is based on establishing, by means of a periodogram analysis of the libratory motion executed by the selenoid satellite, its free periods. The latter depend solely on the ratio of the masses of the Earth and the Moon which can thus be directly calculated. The determination of such periods could be accomplished with considerable accuracy on the basis of straightforward astronomical observation of the position of the selenoid satellite on the celestial sphere. When the above method was originally suggested, the use of artificial selenoid satellites (in the form of large inflated balloons) was contemplated. Quite recently, however, K. Kordylewski, of the Cracow Observatory, has reported the discovery of two (cloud-like) natural selenoid satellites orbiting in the vicinity of the trailing libration centre of the lunar orbit[3]. On the basis of the data communicated by Kordylewski, the amplitude of the libration of the two bodies can be estimated to be of the order of a few degrees, which appears adequate for a determination of the periods as described above. It is hoped that a sufficient number of sufficiently accurate data will be available in the near future, thus permitting application of the proposed method. It should be noted that a complete description of the motion of the selenoid satellites is redundant for the purpose discussed here, since the information required can be extracted from the knowledge of the dependence on time of any appropriate co-ordinate.

E. T. Benedikt

Space Physics Laboratory,
Astro Sciences Group,
Norair Division of Northrop Corporation,
Hawthorne, California.

[1] Benedikt, E. T., and Levin-Cohen, P. S., *Advances in Astronautical Sciences*, **4**, 311 (New York and London, 1958).
[2] Benedikt, E. T., *First Intern. Symp. Analytical Astrodynamics* (Los Angeles, 1961).
[3] Kordylewski, K., *Circ. No. 1760, Intern. Astro. Union* (May 23, 1961).

PHYSICS

Radioactivity in Rainfall during September 1961 following the Russian Nuclear-Weapons Tests of September 1961

The collection and analysis of rainwater are a part of the environmental survey programme of this Laboratory. Simple analysis of the total β-activity, consisting of evaporation and counting the residue, using anti-coincidence low-level techniques, has been carried out over the past two years and specific isotopic analysis has been performed when this was of particular interest, as at the present time.

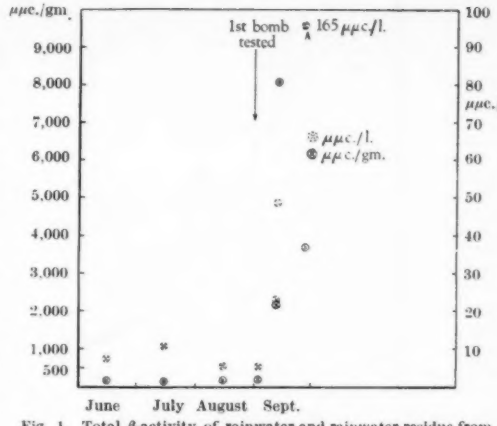


Fig. 1. Total β-activity of rainwater and rainwater residue from June to September 31

From the analyses for total β-activity it is evident that the residue from rain falling after September 2, 1961, that is, following the commencement of the Russian nuclear tests, had an activity which was of a distinctly higher order (up to 8,000 μμc./gm.) than that of previous months rainfall (about 160 μμc./gm.). The activity of the rainwater in this period was correspondingly greater (48·4 μμc./l.) compared with previous months (about 5·1 μμc./l.).

The results of these and subsequent samples are presented in Table 1. The build-up of activity in the rain can be seen from a graphical interpretation of these results (Fig. 1).

Table 1. Total β-Activity of Rainwater and Rainwater Residue

Date of sample collection	Date of sample analysis	Rainwater * (μμc./l.)	Rainwater residue * (μμc./gm.)
1/8/61–31/8/61	12/ 9/61	5·1	157
2/9/61– 4/9/61	5/ 9/61	3·4	188
12/9/61–13/9/61	20/ 9/61	22·7	2,160
13/9/61–14/9/61	18/ 9/61	48·4	8,050
27/9/61–28/9/61	3/10/61	165·0	2,360

* Activity at date of analysis.

In view of the complex nature of the uptake of solid matter during and after cloud formation, both activities per litre of rainwater and per gram of residue have been given. The uncertainty in both these figures is conservatively estimated at ± 20 per cent, so that the increases after September 12 are highly significant.

Specific Isotopic Analysis. To confirm that the excess activity was due to fission products, specific isotopic analysis was carried out for iodine-131. This isotope was chosen partly because of its biological importance, but it was also convenient as a tracer because of its characteristic 0·36-MeV. γ-ray. The half-life of 8·05 days was sufficiently long to permit precise measurement but not long enough to permit iodine contamination from previous tests to cause confusion.

Chemical separation of iodine was made and the resulting samples were counted for β-activity using anti-coincidence methods. The activity was attributed

Table 2. Iodine-131 in Rainwater (Activity)

Date of sample collection	Date of sample analysis	Activity at date of analysis μμc./l. of iodine-131
13/9/61–14/9/61	14/9/61	9·8
14/9/61–18/9/61	18/9/61	95·5 82·0 *
27/9/61	28/9/61	14·2

* By γ-ray spectra analysis.

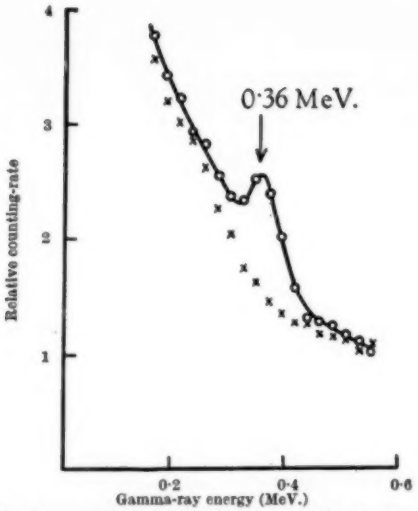


Fig. 2. Gamma spectrum of iodine-131 in rainfall. ○, Source; ×, background

to iodine-131 in view of the comparatively short half-lives of the other iodine fission products. A confirmatory measurement on one of the samples was made by γ-ray spectrometer analysis for iodine-131. Good agreement was obtained, as can be seen in Table 2. The spectrum obtained is shown in Fig. 2.

The confirmatory test of the γ-ray spectrometer on the second sample yielded a result of 82 μμc./l. of iodine-131 compared with 95·5 μμc./l. by β-analysis.

Although these preliminary results indicate clearly the onset of the Russian testing programme, it is impossible at this stage to attempt a more detailed correlation with individual tests.

I would like to thank Mr. R. A. Pope for measuring the γ-ray spectra, and the staff of the Radiological Protection Unit for the preparation and analysis of samples. I would like to express my special thanks to Dr. S. E. Hunt for his helpful advice and comments. Thanks are due also to Dr. T. E. Allibone, director of the Laboratory, for permission to publish this communication.

Keith Boddy

Research Laboratory,
Associated Electrical Industries,
Aldermaston Court,
Aldermaston, Berks.

Nuclear Spin of Protoactinium-231 from Hyperfine Splitting

The nuclear spin of protoactinium was first determined by Schuler and Gollnow in 1934 [1]. They examined the spectrum of protoactinium-231 in the region 4300–5600 Å. and obtained the nuclear spin $I = \frac{3}{2}$ from hyperfine splitting.

More recently the nuclear spin of protoactinium-233 has been determined [2] from paramagnetic resonance, and the nuclear spin of tetravalent protoactinium-231 from an investigation of paramagnetic resonance and hyperfine structure [3]. Both authors obtain $I = \frac{3}{2}$.

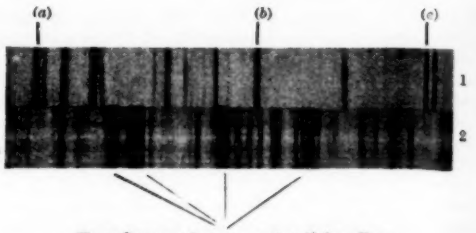


Fig. 1. Protoactinium-231 spectrum with thorium comparison spectrum. 1, Thorium comparison spectrum; 2, protoactinium spectrum; *a*, thorium, 5869·847 Å.; *b*, thorium, 5874·981 Å.; *c*, thorium, 5878·922 Å.

So far, however, the work of Schuler and Gollnow has not been confirmed.

The atomic emission spectrum of protoactinium-231 was recently photographed in the region 4000–9000 Å. using the 21-ft. grating spectrograph at Harwell, and also recorded in the region 0·8–2·5μ. The light source was an electrodeless discharge tube containing about 3 mgm. of protoactinium iodide and excited by microwaves. The spectrum shows pronounced hyperfine structure, the majority of lines being split into four components. This confirms the value $I = \frac{3}{2}$ obtained by previous workers.

E. W. T. Richards
A. Ridgeley
N. J. Atherton
H. S. Wise

United Kingdom Atomic Energy Authority,
Harwell, Didcot, Berkshire.

[1] Schuler and Gollnow, *Naturwiss.*, **22**, 511 (1934).
[2] Marrus, Nierenberg and Winocur, *Nuclear Phys.*, **23**, 1, 90 (1961).
[3] Axe, Stapleton and Jeffries, *Nuclear Phys. Abstr.*, **15**, 3213 (1961).

Rotational Analysis of $^{65}Cu^{35}Cl$ Bands

Although many extensive band systems are known for the CuCl molecule [1], our knowledge of their rotational structure has been meagre. Apart from being heavy, natural cuprous chloride is a mixture of four isotopic species with not very different relative abundances. Very high dispersion and resolution are therefore needed to resolve, even partially, the rotational structure of the bands.

With the availability of compounds containing separated copper and chlorine isotopes, it has become possible to prepare copper chloride with any desired copper and chlorine isotopes. Milligram quantities of $^{65}Cu^{35}Cl_2$ have been thus prepared and the spectrum of $^{65}Cu^{35}Cl$ excited in an electrodeless discharge tube by a 2,450 mc./sec. Raytheon microwave oscillator [2]. All the known band systems of cuprous chloride were obtained with high intensity. The bands were photographed mostly in the fifth order of the Argonne National Laboratory 30-ft. concave grating spectrograph (dispersion 0·3 Å./mm.). Bands for which rotational analysis has been done are given in Table 1. Spectroscopic data obtained from the present work are given in Table 2. All values in this table, except those of r_e, are in cm.$^{-1}$ units.

Table 1

System	Bands analysed
$B - X(^1\Pi - {}^1\Sigma)$	(1,0); (0,0); (0,1)
$C - X(^1\Sigma - {}^1\Sigma)$	(1,0); (0,0); (0,1)
$D - X(^1\Pi - {}^1\Sigma)$	(3,1); (2,0); (1,0); (0,0); (0,1); (0,2)
$E - X(^1\Sigma - {}^1\Sigma)$	(4,1); (3,0); (2,0); (1,0); (0,0); (0,1)
$F - X(^1\Pi - {}^1\Sigma)$	(3,0); (2,0); (1,0); (0,0); (0,1); (0,2)

Table 2. MOLECULAR CONSTANTS OF $^{63}Cu^{35}Cl$

State	T_e	ω_e	$\omega_e x_e$	$\omega_e y_e$	B_e	$\alpha_e \times 10^4$	$q \times 10^3$	$D_e \times 10^7$	r_e (10^{-8} cm.)	Observed transitions Designation	$\nu_{v,0}$
F	25,285·30	384·94	1·65	—	0·1608	9·1	—	1·2	2·148	$F \longrightarrow X$	25,270·1[1]
E	23,074·33	403·08	1·49	−0·03	0·1663	10·8	—	1·0	2·112	$E \longleftrightarrow X$	23,068·25
D	22,970·04	392·94	1·73	—	0·1677	8·4	1·6	—	2·103	$D \longleftrightarrow X$	22,958·83
C	—	—	—	—	0·1691	8·9	—	1·2	2·094	$C \longleftrightarrow X$	20,621·78
B	—	—	—	—	0·1685	9·3	0·7	1·2	2·098	$B \longleftrightarrow X$	20,476·07
X	0	415·29	1·58	—	0·1764	10·0	—	1·3	2·050		

The rotational analysis indicates that the upper states of the five systems are either $^1\Pi$, $^1\Sigma$, etc., as shown in Table 1 or states which 'behave' like these. The Λ-type doubling in the F state is negligible, whereas that in the D and B states is large. This is apparently due to the circumstance that whereas there is no perturbing state near the F level, the levels D and B have each a perturbing state nearby.

In a recent note, Lagerqvist *et al.*[3] have given the results of their rotational analysis of the (1,0), (0,0) and (0,1) bands of the B–X system and the (1,0) band of the C–X system of $^{63}Cu^{35}Cl$ recorded in absorption. The rotational constants of $^{63}Cu^{35}Cl$ calculated from the present $^{63}Cu^{35}Cl$ data are in excellent agreement with those given by them.

R. K. ASUNDI
P. RAMAKOTESWARA RAO

Atomic Energy Establishment,
Trombay,
Bombay.

J. K. BRODY

Argonne National Laboratory,
Argonne,
Illinois.

[1] Herzberg, G., *Spectra of Diatomic Molecules* (D. van Nostrand Co., Inc., New York, 1950).
[2] Ramakoteswara Rao, P., and Brody, J. K., *J. Chem. Phys.*, **34**, No. 8 (1961).
[3] Lagerqvist, Albin, and Lazarova-Girsanof, Vesselina, *Naturwiss.*, **48**, 68 (1961).

Electron Paramagnetic Resonance and Antiferromagnetism in $LaCrO_3$

THE presence of antiferromagnetic ordering in $LaCrO_3$ has been verified before by both neutron diffraction[1] and static magnetic susceptibility[2] techniques. In the former case, a Néel temperature of approximately 320° K. is reported, while the susceptibility results indicate $T_n \approx 295°$ K. Above T_n, $LaCrO_3$ is paramagnetic and hence amenable to investigation by standard electron paramagnetic resonance techniques. We have carried out the experimental resonance investigations over the temperature-range 200–450° K.

The powder was prepared by heating reagent grade Cr_2O_3 and La_2O_3 at 1,600° C. for 2 hr. Our X-ray diffraction results indicate a typical perovskite with small distortions from cubic symmetry. Assuming a cubic structure, we find the lattice parameter to be 3·870 Å. in reasonable agreement with the value 3·874 Å. published in ref. 3.

The pertinent features of our results are summarized in the figures. All data were obtained at 9·1 kMc./s. using a Varian V–4500 electron paramagnetic resonance spectrometer with d.c. magnetic field varied continuously from 0 to 10,000 gauss. The resonances were singly peaked, with g values equal to 1·98, at all observable temperatures.

Our results are consistent with previous resonance investigation of similar compounds[4]. In the antiferromagnetic state anisotropy and exchange fields effectively 'drive' the resonance from the region of observation[5]. Line-widths exhibit the behaviour predicted by the calculations in ref. 6. With antiferromagnetic ordering each Cr^{3+} ion is indirectly coupled to the six nearest similar ions with spin orientation opposite to that of the central ion. Since the spins 'lock' into this configuration, the time-averages of the dipolar fields at a particular spin site approach the instantaneous value. Hence line-broadening results. Extinction of resonance below the transition appears to rule out impurities as a major contributor to line-width.

In the paramagnetic region, it is expected that peak intensities will be proportional to the magnetization and hence the susceptibility[7]. However, it has been argued that peak intensity should depend on both magnetic susceptibility and the existence of some residual short-range order close to, and above, T_n (ref. 8). The transitions observed here are relatively abrupt when compared with similar results for other compounds[8]. This is interpreted as indicating a minimization of short-range order effects above and close to T_n. Hence from the maximum in relative intensity we obtain $T_n \approx 295°$ K. Our numerical agreement with the susceptibility results is, no doubt, fortuitous. The two results are, however, taken to be in approximate agreement. In any event, the present results differ appreciably from that of the neutron diffraction experiments. In this respect it is perhaps significant to note that Jonker[2] prepared his sample in a manner somewhat similar to ours, using the same compounds as starting materials. On the other hand, Koehler and Wollan[1] used as their starting

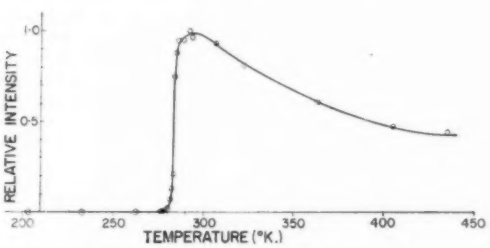


Fig. 1. Relative intensities versus temperature for electron paramagnetic absorption in $LaCrO_3$

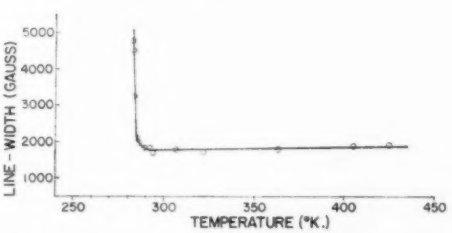


Fig. 2. Line widths, at half-maximum, versus temperature for electron paramagnetic absorption in $LaCrO_3$

materials a lanthanum compound in stoichiometric mixture with the transition metal.

I. Weinberg
P. Larssen

Research Laboratories,
Aeronutronic,
Division of Ford Motor Company,
Newport Beach,
California.

[1] Koehler, W. C., and Wollan, E. O., *J. Phys. Chem. Solids*, 2, 100 (1957).
[2] Jonker, G. H., *Physica*, 22, 707 (1956).
[3] Ruggiero, A., and Ferro, R., *Gazz. Chem. Ital.*, 85, 892 (1955).
[4] Trounson, E. P., Bleil, D. F., Wangsness, R. K., and Maxwell, L. R., *Phys. Rev.*, 79, 542 (1950). Maxwell, L. R., and Mcguire, T. R., *Revs. Mod. Phys.*, 25, 279 (1953).
[5] Nagamiya, T., *Prog. Theor. Phys. (Kyoto)*, 6, 350 (1951). Kittel, C., *Phys. Rev.*, 82, 565 (1951).
[6] Tsuya, N., and Ichikawa, Y., *Phys. Rev.*, 83, 1065 (1951); *Sci. Reports*, Tohoku University, 35, 74 (1951).
[7] Pryce, M. H. L., and Stevens, K. W. H., *Proc. Phys. Soc.*, A, 63, 36 (1950).
[8] Wangsness, R. K., *Phys. Rev.*, 89, 192 (1953).

GEOLOGY

Potassium-Argon Ages at Broken Hill, Australia

We have obtained three results which contribute something to an understanding of the more recent events in the Broken Hill district of New South Wales. Field evidence[1,2] indicates that the main lead lodes lie conformably with gneisses of the Willyama Series, thought to have been formed from sediments of older Precambrian age. These have been strongly folded, the metamorphism and folding having taken place before deposition of the younger Torrowangee Series, which is regarded[3] as equivalent to the Adelaide System of Upper Precambrian age. At some subsequent time, possibly in the early Palæozoic, greenschist facies assemblages were developed (White, A. J. R., personal communication) in the Torrowangee Series, suggesting that temperatures were reached adequate for the loss of a considerable proportion of any argon (if not all) accumulated up to that time[4]. Although there is comparatively little evidence for this heating (Carruthers, D. S., personal communication) in the earlier Willyama rocks, it is inconceivable that they can have been completely unaffected. This inference is supported by our results.

The Broken Hill samples analysed were:

GA 113	Upper granitic gneiss (Formation No. 13), New Broken Hill Consolidated, Ltd.	Age 504 m.y.
GA 114	Sillimanite biotite garnet gneiss (Formation No. 4), No. 21 level, Zinc Corporation, Ltd.	Age 526 m.y.

These results are to be compared with the age determined on a transgressive-type granite (*GA* 212) from Encounter Bay, in the southern part of the Adelaide Geosyncline. The date obtained here, 457 m.y., can be regarded as being related to the more recent events in the Adelaide Geosyncline, thus constituting a possible upper limit to the time of any movement of the Torrowangee at Broken Hill. Thus the Cambrian ages obtained for the undoubtedly older gneisses suggest that the event affecting the Torrowangee happened between 500 and, say, 460 m.y. ago, with the implication that the effect on the older Willyama rocks may not have been sufficiently intense to cause total loss of argon. There is inadequate evidence to relate these results with the two 'model ages' derived from lead isotope measurements by Russell *et al.*[5], of 1,600 m.y. for the conformable leads, and "less than 1,000 m.y." for the event causing the formation of the transgressive Thackaringa-type deposits. Further measurements are in progress, and more complete details of these experiments are to be published elsewhere.

J. F. Evernden

Department of Geology,
University of California,
Berkeley.

J. R. Richards

Department of Geophysics,
Australian National University,
Canberra.

[1] Thomas, W. N., unpublished lecture delivered at A.N.Z.A.A.S., Brisbane, June 1, 1961.
[2] Leslie, R. B., and White, A. J. R., *Trans. Roy. Soc. South Austral.*, 78, 121 (1955).
[3] David, T. W. E., *Geology of the Commonwealth of Australia*, edit. by Browne, W. R. (E. Arnold and Co., London, 1950).
[4] Evernden, J. F., Curtis, G. H., *et al.*, *Amer. J. Sci.*, 258, 583 (1960).
[5] Russell, R. D., and Farquhar, R. M., *Lead Isotopes in Geology* (Interscience, London, 1960).

METALLURGY

Difference between High- and Low-Stress Fatigue

Recent papers by Porter and Levy[1] and Benham and Ford[2] have suggested that the conventional S/N curve is composed of two separate curves, a discontinuity occurring at some particular stress-level. The former authors concluded from a large number of rotating bending tests on copper that the discontinuity occurred at about $\pm$ 21,500 lb./sq. in.; for lower stresses fatigue cracks originating in slip bands; for higher stresses cracks developing from L-shaped nuclei. Kemsley[3] also found that with copper specimens tested at $\pm$ 15,000 lb./sq. in. (giving fracture after long endurances) cracks formed in slip bands and were transcrystalline, while at $\pm$ 25,000 lb./sq. in. (giving fracture with low endurances) slip bands were virtually absent from the specimen and cracks appeared to be intercrystalline. Benham and Ford[2], who tested mild steel in direct stress, found for large stress amplitudes that the specimen gauge-length extended permanently each cycle and a 'necked-out' fracture resulted. They termed this phenomenon 'cyclic creep' and such failures occurred while the stresses corresponded to the upper portion of the composite S/N diagram. For stresses in the lower portion of the S/N diagram typical fatigue fractures occurred and they suggested that, if the fatigue mechanism could be suppressed as the cyclic stress decreased, the upper curve might flatten out to a 'cyclic creep limit'.

Low-endurance fatigue tests have been carried out by many workers, the usual procedure being to apply strain cycles rather than stress cycles of constant amplitude; the work of Coffin[4] is typical. Coffin suggested the results of such tests could be represented by the equation:

$$N^{\frac{1}{2}} \varepsilon_p = C$$

(where N = number of cycles to failure, ε_p = cyclic-plastic strain amplitude and C = material constant). The endurances over which this relationship applies vary with material. Coffin found for copper it was about 10^2 cycles, for aluminium about 10^5 cycles.

On the other hand, when the cyclic stresses are in the vicinity of the fatigue limit, no relationship exists between the width of the hysteresis loop and life of specimen. For such tests, metallographic examination of the specimen surface shows, in general, that slip bands form early in the test from which micro-cracks eventually develop. Indeed, both Hempel[5] and Wadsworth[6] have observed that, with mild steel, cracks may form in slip bands at stresses just below the fatigue limit. Crack initiation may thus be considered as a consequence of surface roughening within broad slip bands[7,8].

Holden[9] has proposed a mechanism of failure, distinct from that responsible for surface crack initiation, occurring when the material is subjected to cyclic strains such as those occurring in the material ahead of a growing fatigue crack. From examination, by means of an X-ray micro-beam technique, of the fractured faces of a material through which a crack had grown and of plain specimens subjected to large cyclic-plastic strains, he found for mild steel and aluminium the structure consisted of well-defined sub-grains. The sub-grains became progressively more misorientated as the test proceeded and he suggested that cracks could form in the sub-grain boundaries.

Sub-grains have been observed metallographically on the surface of fatigue specimens by, for example, Forsyth[10] and Jacquet[11], although, in their case, the sub-grain structure did not contribute to failure presumably because the cyclic stress was insufficient to misorientate the sub-grains through sufficiently large angles, and failure arose from surface roughening as mentioned above.

It could thus be argued that, when plain specimens are subjected to such nominal stresses that failure occurs in a small number of cycles, failure is due to the formation of sub-grains in the bulk of the specimen and cracks forming in the sub-grain boundaries. In such cases the life of the specimen is related to the plastic strain amplitude; indeed, Holden suggested that the random movement of dislocations into arrays leads to a relationship of the type suggested by Coffin. As the nominal stress amplitude is reduced a stress-level will be reached at which either the sub-grain structure does not form or, if sub-grains do form, they are unable to misorientate through sufficiently large angles to cause cracking. This stress-level may be considered as the 'true plain fatigue limit' although, of course, its value is much higher than the conventional plain fatigue limit. For a plain specimen to fail at lower stress-levels it is necessary for surface roughening phenomena to produce surface grooves or cracks. The sub-grain structure will then form at the groove root due to the local strain concentration and the crack may grow across the specimen. Whether or not cracks of macroscopic dimensions formed in notched specimens grow has been discussed by me[12]. It is not unreasonable to suppose that the behaviour of micro-cracks in surface grooves will be similar. Thus, at stresses below the 'true fatigue limit' a specimen fails because surface roughening phenomena effectively turn the plain specimen into a notched one. The life and nominal cyclic-plastic strain are now no longer subject to the same relationship as that which applies when stresses are above the 'true plain fatigue limit'. The cyclic stress below which the specimen will not break (the conventional plain fatigue limit) is that value of the cyclic stress which is just unable to produce surface grooves or cracks to a sufficient depth to enable them to grow.

This communication is published by permission of the Director of the National Engineering Laboratory.

N. E. Frost

Materials Group,
National Engineering Laboratory,
East Kilbride, Glasgow.

[1] Porter, J., and Levy, L. C., *J. Inst. Metals*, **89**, 86 (1960).
[2] Benham, P. P., and Ford, H., *J. Mech. Eng. Sci.*, **3**, 119 (1961).
[3] Kemsley, D. S., *J. Inst. Metals*, **85**, 417 (1957).
[4] Coffin, L. F., *Internal Stresses and Fatigue of Metals*, 353 (Elsevier, 1959).
[5] Hempel, M., *Int. Conf. Fatigue, Inst. Mech. Eng.*, 543 (1956).
[6] Wadsworth, N. J., *Phil. Mag.*, **6** 397 (1961).
[7] Wood, W. A., *Phil. Mag.*, **3**, 692 (1958).
[8] May, A. N., *Nature*, **185**, 303 (1960).
[9] Holden, J., *Phil. Mag.*, **6**, 547 (1961).
[10] Forsyth, P. J. E., *Int. Conf. Fatigue, Inst. Mech. Eng.*, 535 (1956).
[11] Jacquet, P. A., *Int. Conf. Fatigue. Inst. Mech. Eng.*, 506 (1956).
[12] Frost, N. E., *J. Mech. Phys. Solids*, **9**, 143 (1961).

CRYSTALLOGRAPHY

Lattice Rotation at Slip Band Intersections

Intersection of a slip band by an active slip band of different slip direction results in the formation of a step in the first band. Slip band intersections in magnesium oxide crystals suggested that the distortion of the intersected band was not merely a shear due to lattice translation; this was supported by the observation that subsequent widening of the intersected band (as described by Gilman and Johnston[1]) added to it a margin that followed the step. This indicated that there was a change of lattice orientation at the intersection. Imaging X-ray photographs of the Berg–Barrett[2,3] type confirmed the change of lattice orientation. Fig. 1 shows a compressed magnesium oxide crystal, afterwards etched to reveal dislocations; the dislocation etch-pits make the bands appear dark. Fig. 2 is an X-ray image of the same crystal; the slip bands appear dark because of the reduction of primary extinction. The slip band inter-

Fig. 1. Etched surface of compressed magnesium oxide crystal; reflected light

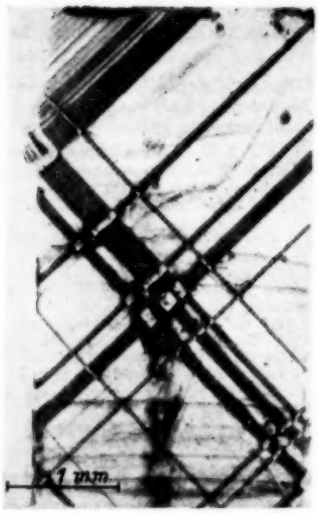


Fig. 2. X-ray back reflexion image of same surface as in Fig. 1; copper K_α radiation; reflexion from (210) plane

sections are white, and the reflexions from them can be found in more or less displaced positions, showing that there is a macroscopic change of orientation of the lattice at the intersections. Such a change can arise in the way shown in Fig. 3. Since the intersection is full of edge dislocations of both slip directions [110] and [1$\bar{1}$0], and the shear stress is the same in both slip systems, the shear strain in the active slip band may be due to slip and kinking[4,5] (= slip on the planes of the intersecting band) in the area of intersection. In Fig. 3 all strain in the intersection is assumed to have been the result of kinking.

In Fig. 3, the parallelogram of the intersection is bounded by edge dislocations arranged in the same sense of rotation, because the dislocations of th intersecting band have arrived from outside, those of the intersected band (the kink dislocations) from inside the parallelogram. Evidently, the lattice rotation due to conversion of slip into kinking at the intersection has the direction shown by X-ray asterism of the microscopic type (asterism of the macroscopic type is due to bending of the slip lamellæ[6]; it is a different phenomenon). Conversion of slip to kinking is possible whenever slip bands with different slip directions intersect; it may also occur between parallel slip bands in sodium chloride[7], and it seems to be the cause of microscopic asterism generally.

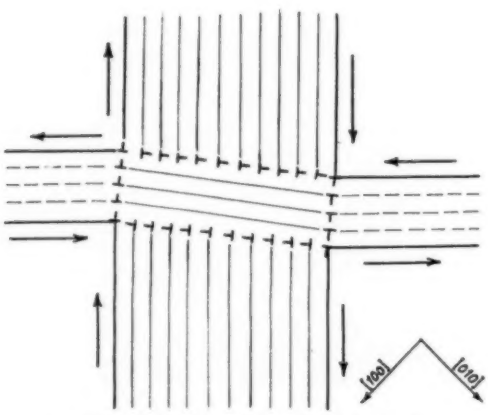


Fig. 3. Kinking at the intersection of slip bands

The array of dislocations forming the low-angle boundary of the intersection is an obstacle to further slip and a possible cause of strain hardening in the post-easy-glide region. Kink conversion, therefore, may perform the same task in the theory of strain hardening as Lomer–Cottrell dislocations, with the advantage of being a more general and observationally less elusive process. Fig. 3 indicates that it is a reversible process which may be connected with the Bauschinger effect and associated reversal phenomena. Since the kink-conversion areas oppose slip in one direction more than in the opposite one, they may play a part in fatigue and extrusion phenomena[8]. The stresses due to kink-conversion may lead to crack formation in several ways, explaining the observations of Stokes, Johnston and Li[9] which could scarcely be attributed to crack nucleation by reactions between individual dislocations.

This work was sponsored by the Wright Air Development Division through the Armour Research Foundation.

A. S. Argon
E. Orowan

Massachusetts Institute of Technology.
Cambridge, Massachusetts.

[1] Gilman, J. J., and Johnston, W. G., *Dislocations and Mechanical Properties of Crystals*, edit. by Fisher *et al.*, 116 (J. Wiley and Sons, New York, 1957).
[2] Berg, W., *Naturwiss.*, **19**, 391 (1931); *Z. Kristallog.*, **89**, 286 (1934).
[3] Barrett, C. S., *Trans. Amer. Inst. Mech. Eng.*, **161**, 15 (1945).
[4] Mügge, O., *Neues Jahrb. Miner.*, **1**, 71 (1898).
[5] Orowan, E., *Nature*, **149**, 643 (1942).
[6] Orowan, E., and Pascoe, K. J., *Nature*, **148**, 467 (1941).
[7] Brilliantow, N. A., and Obreimow, I. W., *Phys. Z. Sowjetunion*, **5**, 587 (1934).
[8] Forsyth, P. J. E., and Stubbington, C. A., *J. Inst. Metals*, **83**, 395 (1955).
[9] Stokes, R. J., Johnston, T. L., and Li, C. H., *Phil. Mag.*, **3**, 718 (1958).

CHEMISTRY

Requirements of an Ice Nucleus

The striking similarity between the crystal lattices of silver iodide and ice led Vonnegut[1] to the conclusion that the efficiency of a substance as an ice nucleator depended on the closeness of fit between the ice lattice and the lattice of the nucleating substance. This view persisted for some years, but it has become increasingly apparent[2] that nucleation is not governed by lattice spacing only and that a good nucleator must also possess some other characteristic. By taking into account the changes in entropy involved in growing ice on a polar substrate, Fletcher[3] has predicted that the second necessary characteristic of a good nucleator is that exactly half the water molecules comprising the layer immediately adjacent to the nucleating surface should have a proton directed towards this surface. As a first approximation this requires that a uni-univalent nucleator such as silver iodide should present a surface comprised of equal numbers of positive and negative ions; thus the surface should be at its isoelectric point, and any departure from this point due to the adsorption of

potential-determining ions would be expected to decrease the nucleating efficiency.

In what follows we present what we believe to be the first experimental demonstration that ice nucleation is indeed a function of the surface charge of the nucleating substrate.

The surface charge of silver iodide particles in contact with an aqueous solution is readily controlled by the concentration of silver ions and iodide ions in the solution[4]. Therefore the freezing point of a small droplet of such a solution in contact with silver iodide particles reflects the effect of surface charge, providing that there is no interfering electrolyte effect, and that each droplet contains a uniform quantity of silver iodide. To meet these requirements, monodisperse sols of constant particle size and concentration were prepared by diluting, with water, a saturated solution of silver iodide in 0·2 *M* potassium iodide. A uniform deposit of sol particles was centrifuged on to glass coverslips which had previously been rendered hydrophobic by treatment in chlorsilane vapour. The particle-coated slips were washed thoroughly in water, sprayed with the required solution by means of a spinning disk atomizer, transferred to a microscope cold stage, cooled at a rate of 2 deg. C. per min., and the freezing point of each droplet noted. To avoid undue scatter the freezing point of only the droplets between 30μ and 60μ in diameter was recorded, the average number of particles per 50μ droplet being 200. In each experiment about 50 droplets were observed, and generally all the droplets froze within a range of 4 deg. C.

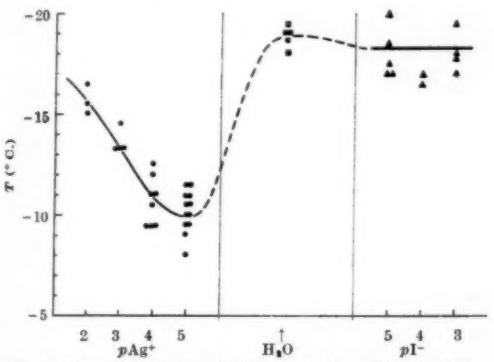


Fig. 1. The variation of T with the composition of the droplets. ●, Silver nitrate solutions; ▲, potassium iodide solutions; ■, distilled water

Fig. 1 shows how T, the temperature at which 50 per cent of the droplets had frozen, varied with the composition of the droplets.

It is apparent that, as Fletcher's theory would suggest, nucleation is most efficient in the vicinity of the isoelectric point (10^{-6} *M* silver nitrate[4]) and that quite extreme supercooling is required when the surface is charged either positively or negatively. That iodide ion should be the more effective in this regard is consistent with the known tendency of silver iodide to adsorb iodide ions in preference to silver ions.

These results are unaltered by the presence in all solutions of 10^{-2} *M* potassium nitrate, therefore the variations in T are not due to changing ionic strength.

It is of considerable practical importance that the negative charge which silver iodide acquires in pure water is sufficient to hinder nucleation. This suggests a new approach to the search for the best cloud-seeding substance—namely, that the substance should be at the isoelectric point under the conditions prevailing within a super-cooled cloud droplet.

G. R. Edwards
L. F. Evans

Division of Physical Chemistry,
Commonwealth Scientific and
Industrial Research Organization,
Melbourne.

[1] Vonnegut, B., *J. App. Phys.*, **18**, 595 (1947).
[2] Bryant, G. W., Hallet, J., and Mason, B. J., *J. Phys. Chem. Solids*, **12**, 189 (1959).
[3] Fletcher, N. H., *J. Chem. Phys.*, **30**, 1476 (1959).
[4] Kolthoff, I. M., and Lingane, J. J., *J. Amer. Chem. Soc.*, **58**, 1528 (1936).

Evaluation of Boundary Layer Thickness in Electrolytic Systems from Decay of Anode Concentration Polarization

The boundary layer produced in the vicinity of a dissolving anode in an electrolytic system is maintained by a supply of ions from the electrode, which supply balances the migration away from the layer due to the combined effects of electrical transport, diffusion and convexion. On interruption of current, the excess concentration of the existing boundary layer is reduced by convexion and diffusion into the bulk of the electrolyte. The convexion conditions may be very complex since a natural convexion mode occurs as a result of changes in solution density in the immediate vicinity of the electrode.

Two extreme limiting conditions may be established for the decay of the excess concentration in the boundary layer on current interruption. First, it may be assumed that there is zero relative motion between the fluid of the layer and that of the bulk electrolyte. This 'pure diffusion' extreme is analogous to the levelling of a concentration excess from a zone within a solid to the remainder of the solid. Secondly, it may be assumed that 'perfect stirring' corresponds to the decay of an excess concentration within a zone with no accompanying change in the spatial dimension of the zone, together with a continual maintenance of the bulk concentration at the outermost extremity of the zone. In the 'perfect stirring' case, natural convexion is considered to contribute negligibly to the establishment of the thickness of the boundary layer, this layer being chiefly a function of externally imposed stirring conditions.

Intermediate to the extreme conditions must lie 'mixed' control of collapse of the boundary layer by both stirring and natural convexion in addition to the ever-present diffusion.

The concentration contour of the boundary layer may be approximated by a straight line tangent to the slope of the actual contour at the electrode ($x = 0$), thus denoting an effective boundary layer thickness at the point of intersection of this line with the bulk concentration.

For any given effective boundary layer thickness δ, the concentration (strictly, activity) at $x = 0$ is given, where zero time occurs at current interruption for 'pure diffusion' as:

$$\frac{C-C_b}{C_0-C_b} = \operatorname{erf}\frac{1}{2\tau^{1/2}} + \frac{2}{\pi^{1/2}}\tau^{1/2}\left[e^{-1/4\tau}-1\right] \qquad (1)$$

and for 'perfect stirring' as:

$$\frac{C-C_b}{C_0-C_b} = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\,e^{-\frac{(2n+1)^2\pi^2\tau}{4}} \qquad (2)$$

where $\tau = Dt/\delta^2$; D = diffusion coefficient of ionic species, cm.^{2} sec.$^{-1}$; t = time after current interruption, sec.; δ = effective boundary layer thickness (cm.); C_0 = initial concentration of ionic species (involved in the electrode reaction) at the electrode-electrolyte junction ($x = 0$); C = concentration of ionic species at $x = 0$, $t > 0$; C_b = bulk concentration of ionic species in electrolyte.

Equations 1 and 2 are re-arranged forms of solutions to related problems in heat conduction[1]. Equation 2 may be rendered in an alternative form which is useful for computation when τ is small, namely:

$$\frac{C - C_b}{C_0 - C_b} = 1 - 2\tau^{1/2} \sum_{n=0}^{\infty} (-1)^n \left\{ \frac{1}{\pi^{1/2}} e^{-n^2/\tau} - \right.$$
$$- \frac{n}{\tau^{1/2}} \left[1 - \operatorname{erf} \frac{n}{\tau^{1/2}} \right] - \frac{1}{\pi^{1/2}} e^{-(n+1)^2/\tau} +$$
$$\left. + \frac{n+1}{\tau^{1/2}} \left[1 - \operatorname{erf} \frac{n+1}{\tau^{1/2}} \right] \right\} \qquad (3)$$

For very small τ, equation (1) may be approximated as:

$$\frac{C - C_b}{C_0 - C_b} = 1 - \frac{2}{\pi^{1/2}} \tau^{1/2} \qquad (4)$$

Similarly, equation 3 may be reduced in form for small τ since all terms become zero for $n > 0$. The result is identical with equation 4. Thus, for small τ, the time behaviour of concentration at $x = 0$ is essentially independent of conditions at the outermost part of the boundary layer, that is, irrespective of the presence or absence of stirring.

Because of the equality of the solutions given by equations 1 and 2, 3 for small τ, it is suggested that this result may be utilized for the evaluation of boundary layer effective thickness in electrolytic systems. This may be accomplished by analysis of the decay of concentration polarization upon current interruption. By the Nernst relation:

$$\frac{C - C_b}{C_0 - C_b} = \frac{\exp[ZF\eta/RT] - 1}{\exp[ZF\eta_0/RT] - 1} \qquad (5)$$

where η_0 = concentration polarization at time zero; η = remaining polarization at $t > 0$; F = Faraday constant; R = gas constant; T = °K; Z = number of electrons in the electrode reaction. The approximation to equation 1 given in equation 4 is valid provided:

$$\operatorname{erf} \frac{1}{2\tau^{1/2}} \approx 1 \text{ and } e^{-1/4\tau} \approx 0$$

Arbitrarily choosing erf $1/2\tau^{1/2} = 0.99$ yields an upper limit of $\tau = 0.075$, whereas $e^{-1/4\tau} = 0.01$ sets a limit of $\tau = 0.054$. Taking a limit of $\tau = 0.05$ together with $D = 10^{-5}$ cm.^{2}/sec., it may readily be seen that for the very thin boundary layers encountered in stirred systems ($\delta \approx 10^{-3}$ cm.), the overvoltage measuring device should be able to resolve time to better than 5 msec., suggesting an oscilloscopic technique. For thicknesses of about 0.5 mm., encountered in natural convexion systems, time resolution need only be better than about 10 sec., which is well within the range of chart recorders.

In the dimensionless time $\tau = 0.05$, the concentration (activity) fraction has dropped from unity to about 0.75. The fractional decay of polarization depends on the initial value before current interruption, according to equation 5. At 25° C. and for $z = 2$, an initial polarization of 30 mV. will have decayed to about 14.6 mV. in the interval $0 < \tau < 0.05$. From the relationship of equation 5 with equations 1–4, it is clear that for any hydrodynamic conditions, such as stirring or its absence, all anode concentration polarization data can be normalized according to equation 5 and fitted to a master curve (equation 4) provided the real time-scale (in sec.) is multiplied by D/δ^2. Since D is generally known, a graphical method may be used to cause a plot of the function of η, η_0 given in equation 5 versus time to be superimposed on a master plot the abscissa of which is rendered in dimensionless time τ, such as that given by equation 4 for $0 < \tau < 0.05$.

From equation 4, it may be seen that the time required to attain a given fractional decay varies as the square of the boundary layer thickness δ, indicating that the proposed graphical method will permit a good resolution of the actual magnitude of δ. Equations 1–4 may also prove useful as a means for separating concentration polarization from other types of overvoltage.

Work is in progress to prepare analytical solutions similar to equations 1 and 2 for a concentration contour which is better than the linear approximation for actual systems. Functional tables for equations 1 and 2 over a wide range of τ and tables of the general term of equation 5 are in preparation.

This work was supported by the U.S. Office of Naval Research.

Leonard Nanis

Stanley-Thompson Laboratories,
Henry Krumb School of Mines,
Columbia University,
New York 27.

[1] Carslaw, H. S., and Jaeger, J. C., *Conduction of Heat in Solids*, 54, 97 (Oxford Univ. Press, 1959).

Pyrolytic Carbon Formation from Carbon Suboxide

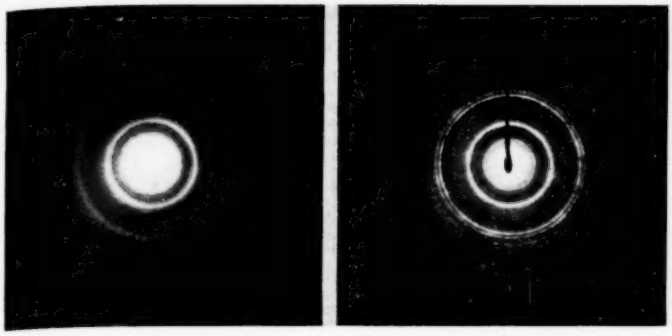
Almost all the extensive work done on the formation of pyrolytic carbon films has consisted of the decomposition of a hydrocarbon on non-metallic substrates like porcelain or artificial graphite. Work is in progress in our laboratory investigating the kinetics and mechanism of carbon film formation on glazed porcelain from carbon suboxide (C_3O_2)[1]. In the course of this work, it was of interest to look briefly, initially, at the effect of some different substrates on the qualitative rate of carbon formation and more particularly on the nature of the film formed.

Very pure C_3O_2, entrained in pre-purified helium, was pyrolysed at 713° C. for about 3 hr. over porcelain, electrolytic nickel and copper foils, and reagent grade platinum foils. The concentration of C_3O_2 at the pyrolysis temperature was about 6×10^{-4} mole/l. Tenacious and hard carbon films were obtained, with the rate of film formation on nickel being roughly four times that on the other substrates.

The carbon films were separated from the metal substrates by acid treatment prior to studying their structure by transmission electron diffraction and

Table 1. Spacings of (*hkl*) Lines of Film Carbon deposited from C_3O_2 on to a Nickel Substrate at 713° C.

(*hkl*) Graphite	d_g, A	d_{fc}, A	Intensity of experimental halos
002	3·370	3·370	medium
100	2·102	2·091	broad ring with very
101	2·036		strong intensity
110	1·232	1·230	very strong
112	1·150	1·155	strong
200	1·063	1·060	medium
210	0·804	0·799	medium
310	0·709	0·704	medium



a *b*

Figs. 1 *a* and *b*. Transmission electron diffraction patterns of film carbon deposited from C_3O_2 on to a substrate at 713° C.; *a*, copper substrate; *b*, nickel substrate

transmission electron microscopy. 50-kV. electrons, having a beam width of 10μ, were used. Fig. 1 (*a*) shows the diffraction pattern for the film carbon resulting from deposition over a copper substrate. Diffuse halos, corresponding to the (10) and (11) reflexions of a polycrystalline, turbostratic carbon[2], are observed. The absence of a strong (002) reflexion when the incident electron beam is normal to the film surface is indicative of preferred orientation of the basal planes parallel to this surface. Diffraction patterns very similar to Fig. 1*a* were obtained from the film carbon deposited on a porcelain or platinum substrate.

On the other hand, the diffraction pattern (Fig. 1*b*) of the film carbon deposited on the nickel substrate is characteristic of a material of substantial crystallite size, in which the crystallites have extensive three-dimensional ordering. Table 1 compares the *d*-spacings for the film carbon (d_{fc}) with comparable spacings calculated from the graphite lattice (d_g). Similar diffraction patterns for carbon films deposited on porcelain, copper, or platinum are obtained only when the separated films are ultimately heated in excess of 2,000° C.

Fig. 2 is an electron micrograph of the carbon film deposited on nickel. Variations in the thickness of the films are evident. In addition, the presence of small, white circular spots is noted. It is suggested that these spots were formed by the removal by acid treatment of inclusions of nickel and/or nickel carbide which were originally present, but this has not been verified. From work on the interaction of evaporated carbon films with a similar nickel substrate on subsequent heat treatment, it is known that substantial inter-diffusion of nickel and carbon occurs at 500° C. and above (Banerjee, B. C., and Walker, P. L., unpublished results).

These results clearly suggest that more attention might be paid to the effect of the substrate in work on the formation of pyrolytic carbon films. In particular, substrates of nickel and iron are of interest. They are highly active catalysts for the decomposition of carbonaceous gases, apparently because of the incomplete filling of their 3*d* bands. In addition, they markedly increase the crystallinity of the carbon formed as a decomposition product. The effect of nickel is clearly evident from this work. The effect of iron, both on rate of formation and structure of carbon, has been previously shown for carbon monoxide decomposition[3].

We thank the Atomic Energy Commission, contract No. *AT*(30-1)-1710, for support of this work.

B. C. Banerjee
T. J. Hirt
P. L. Walker, jun.

Fuel Technology Department,
The Pennsylvania State University,
University Park, Pennsylvania.

[1] Hirt, T. J., and Palmer, H. B., *Abstr. Fifth Biennial Conf. Carbon*, 9 (June 19-23, 1961).
[2] Biscoe, J., and Warren, B. E., *J. App. Phys.*, **13**, 364 (1942).
[3] Walker, jun., P. L., Rakszawski, J. F., and Imperial, G. R., *J. Phys. Chem.*, **63**, 133, 140 (1959).

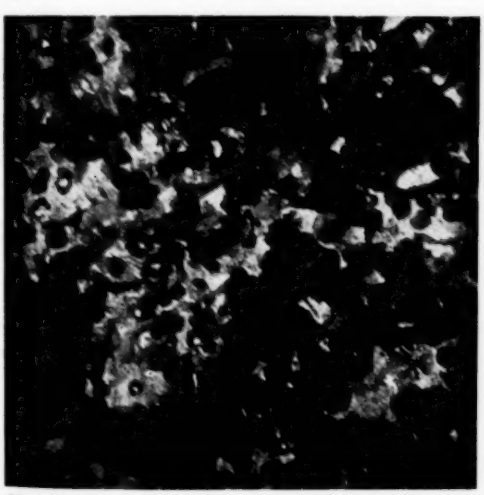


Fig. 2. Transmission electron micrograph of film carbon deposited from C_3O_2 on to a nickel substrate at 713° C. (× *c.* 4,700)

BIOCHEMISTRY

Changes in Adenine Nucleotides in the Muscles of Some Marine Invertebrates

Hitherto it has been generally accepted that most vertebrate muscles contain an enzyme specific for the deamination of 5′-adenylic acid (AMP) which is formed principally from adenosine triphosphate (ATP). On the other hand, in the case of invertebrates the presence of such an enzyme has been questioned[1].

Recently we have studied the changes in adenine nucleotides in the muscles of some marine invertebrates (prawn, squill, crab, squid, and some sea shells), and observed that those of prawn, squill, and crab contain certain amounts of 5′-inosinic acid (IMP) in addition to AMP, while in others AMP alone is accumulated.

Chopped muscles of each animal were stored in a glass-covered dish at an ambient temperature of − 5° C. in order to minimize bacterial effects. Two gm. of muscle were ground by hand in a mortar in 20 ml. of cold, 4 per cent perchloric acid solution. The extract was filtered and 10 ml. of the filtrate were used for

Table 1. Purine Compounds in the Muscles of Some Marine Invertebrates (μmol./gm. of muscle)

Species	Time (days) elapsed	Inosine and hypoxanthine	AMP	IMP	ADP	ATP	Total	Muscle
Carp	0	0·01	0·01	1·06	1·41	2·94	5·43	Dorsal
Cyprinus carpio L.	3 (hr.)	0·10	0·24	4·01	0·60	0·25	5·20	
Squill	0	0·46	0·94	0·50	1·24	3·84	6·93	Abdominal
Squilla oratoria De Haan	6	2·12	3·66	0·75	0·29	0·17	6·99	
Prawn	0	0·00	0·94	0·00	2·09	6·61	9·64	Abdominal
Pandalus hypsinotus Brandt	28	6·14	1·31	1·61	0·17	0·18	9·46	
Crab	0	0·00	0·29	0·00	1·75	5·87	7·91	Leg
Erimacrus isenbeckii	35	1·14	3·47	1·98	0·46	0·35	7·40	
Squid *Ommastrephes sloani*	0	0·01	0·55	0·00	1·53	7·48	9·57	Mantle
pacificus Steenstrup	20 (hr.)	1·35	4·70	0·00	2·89	0·83	9·77	
Surf-clam	0	0·00	0·56	0·00	1·03	3·09	4·68	Foot
Spisula	3	0·31	2·96	0·00	0·95	0·42	4·64	
sachalinensis	0	0·00	0·50	0·00	1·10	4·15	5·75	Adductor
Schrank	2	1·08	2·84	0·00	1·12	0·70	5·74	
Scallop	0	0·00	1·56	0·00	1·18	3·48	6·22	Adductor
Pecten yessoensis Jay.	1	2·99	1·52	0·00	1·17	0·92	6·60	
Arkshell	0	0·00	0·41	0·00	1·19	5·72	7·32	Foot
Anadara	10	0·19*	5·74	0·00	0·66	0·51	7·10	
broughtoni	0	0·00	2·23	0·00	3·50	4·15	9·88	Adductor
(Shrenk)	10	0·42*	7·28	0·00	1·38	0·48	9·56	
Abalone	0	0·00	0·20	0·00	0·59	3·48	4·27	Foot
Haliotis discus hannai	45	0·00	3·04	0·00	0·92	0·30	4·26	

Inosine and hypoxanthine fraction is expressed as inosine. * Expressed as adenine.

analysis. Nucleotides, nucleosides, and bases were separated and determined by the hydrochloric acid gradient elution system on 'Amberlite *IRA*-400' or by paper chromatography. The details of the system have been described previously[2]. In Table 1 the concentrations of the compounds found to be present after various time-intervals are shown.

From these results it can be seen that ATP decreased during storage, with rapid accumulation of IMP in the dorsal muscle of carp, while in the case of squill, prawn, and crab IMP was found in small quantity in living muscles and then gradually accumulated. Furthermore, in other muscle samples AMP alone was found in living or post-mortem muscles.

The rates of disappearance of ATP and of ADP were very rapid for squid and were comparable with those for fish, but the other animals tended to require more time for the onset of post-mortem changes. Especially, the changes in the muscle of abalone were very slow. AMP accumulation continued for a long time. Adenosine, inosine that might be formed and the purine bases, adenine and hypoxanthine, that would be released in the hydrolysis or phosphorolysis of nucleosides could not be found in abalone muscle even after 45 days.

Further interesting aspects of the changes are the formation of inosine and hypoxanthine from AMP of squid, surf-clam, and scallop. Inosine was principally found in the muscles of surf-clam and of scallop while hypoxanthine was found in squid.

The occurrence of these compounds might be considered to prove the existence of the deamination of adenosine or adenine. The activity of adenosine deaminase has already been recognized in squid muscle[3]. On the other hand, according to Florkin[4], invertebrates are reported to contain adenase but no adenosine deaminase. If the existence of adenosine deaminase is ascertained in the muscles of surf-clam and of scallop, as in the case of squid, the new evidence will be accepted to support the idea of the deamination mechanism of adenosine in the muscle of invertebrates. This matter is under consideration.

Ken-ichi Arai
Tsuneyuki Saito

Faculty of Fisheries,
Hokkaido University, Hakodate, Japan.

[1] Conway, E. J., and Cooke, R., *Biochem. J.*, **33**, 479 (1939).
[2] Saito, T., and Arai, K., *Arch. Biochem. Biophys.*, **73**, 315 (1958).
[3] Saito, T., Arai, K., and Tanaka, T., *Bull. Fac. Fish. Hokkaido Univ.*, **9**, 121 (1958).
[4] Florkin, M., in *Biochemical Evolution*, edit. by Morgulis, S. (Acad. Press, New York, 1949).

Effect of Charcoal on the Behaviour of Adenine Nucleotides in Ion-Exchange Chromatography

Charcoal has been used widely for selective adsorption of nucleotides from reaction mixtures and tissue preparations. In experiments reported here three commercially available grades were all found to adsorb quantitatively and to elute reasonably satisfactorily the mono-, di- and tri-phosphates of adenosine; these charcoals were 'Norit', two batches of purified, activated charcoal supplied by Hopkin and Williams, Ltd., and a gift sample of 'Ultrasorb *ZX*' from British Carbo Norit Union, Ltd.

For each trial, between 5 and 10 mgm. of the sodium salts of the individual nucleotides were dissolved together, the solution diluted to about 250 ml. and brought to *p*H 2 with *N* hydrochloric acid. About 4 gm. of charcoal were added, and the mixture stirred vigorously for 15 min. in an ice bath. Under these conditions the nucleotides were quantitatively adsorbed, as indicated both by optical density of the filtrate at 260 mμ and by the orcinol test. The charcoal was then washed with water to neutrality and eluted, under suction on a filter funnel, with several lots of 0·5 per cent ammonia in 25 per cent ethyl alcohol. Using a total eluant volume of 250–300 ml., 80–95 per cent recovery of the nucleotides was obtained. The ammoniacal eluate was subjected directly to ion-exchange chromatography, using the resin in the chloride form, a column of size 1·5 × 3·0 cm., and the elution system of Cohn and Carter[1]. Elution was followed by absorption measurements at 260 mμ of the individual fractions, by pentose estimations, and also (for ADP and ATP) by estimating the 'labile phosphate' liberated by 15 min. hydrolysis at 100° C. in 1·0 *N* hydrochloric acid. The summarized results are as follows.

Provided they had not previously been in contact with charcoal, mixtures of the three adenosine phosphates behaved normally as regards the order and position of elution of their constituents; in other words, they behaved as described by Cohn and Carter (Fig. 1*a*). But after being adsorbed and eluted from any of the charcoals tested, no nucleotide material was removed from the column by the AMP eluant. The ADP eluant, on the other hand, removed quantitatively both the AMP and the ADP, but as a single peak the elution position of which was somewhat behind that normal for ADP (Fig. 1*b*). After suitable

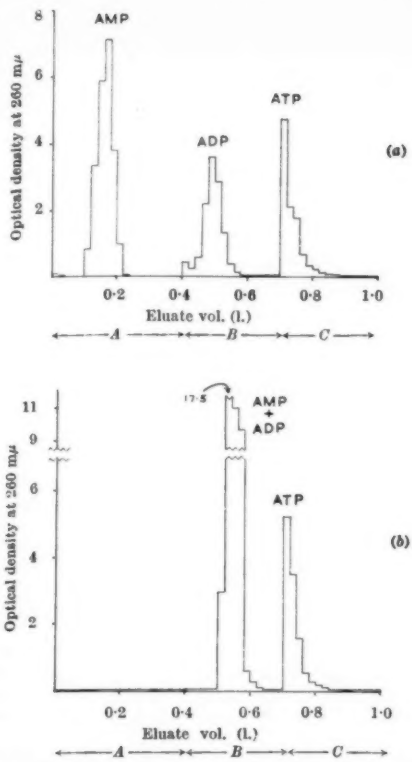


Fig. 1. Effect of charcoal on the chromatographic behaviour of AMP, ADP and ATP. (*a*) Direct chromatography of authentic nucleotides; (*b*) chromatography of nucleotides after charcoal treatment. 'Dowex'-1-chloride, *X* – 10, 2–400 mesh. Column size, 1·5 cm. × 3·0 cm. Eluants: *A*, 0·003 *N* HCl; *B*, 0·02 *N* NaCl + 0·01 *N* HCl; *C*, 0·2 *N* NaCl + 0·01 *N* HCl

dilution (to about twice the volume), and adjustment to *p*H 8–9, the mixed peak was applied to a second column and re-eluted. The two nucleotides then usually separated satisfactorily (Fig. 2), although with the charcoal 'Ultrasorb *ZX*' passage through a third column was needed to obtain full resolution; in this particular case the AMP eluant was used at double its usual strength, but it seems likely that normal strength eluant would have achieved the same result. After adsorption and release of a nucleotide mixture from 'Ultrasorb *ZX*', unsuccessful attempts were made to resolve it on a single resin column by using eluants of increased strength in the first step. Double strength AMP eluant was wholly ineffective, but ADP eluant at half its usual strength removed a peak; this, however, did not consist of AMP alone, since it contained acid-labile phosphate.

AMP was eluted normally when applied alone to a resin column in the ammonia–ethanol solvent without coming into contact with charcoal, whereas, if applied in a quantity of the solvent which had previously been used to wash charcoal (the activated, purified sample), it was no longer eluted with the AMP eluant, but came off only in the position usual for ADP. Prolonged preliminary washing of charcoal with (*a*) 0·5 *M* ethylenediaminetetraacetic acid (sodium salt), (*b*) 0·1 *N* hydrochloric acid, or (*c*) the ammonia–ethanol eluant, did not eliminate its influence on the chromatographic behaviour of AMP afterwards adsorbed on it. On evaporation, ammonia–ethanol washings from charcoal left a slight, unidentified solid residue.

These results point to a need for caution in the selection and use of charcoal for nucleotide adsorption, particularly if subsequent resin chromatography is contemplated. None of the samples proved satisfactory, but some were worse than others. The observations indicate very strongly the presence in charcoal of a substance which induces AMP to behave anomalously on chloride resin. Although the substance is soluble in ammoniacal alcohol, even prolonged washing with many changes of the solvent, which continued to extract the substance, did not render the charcoal innocuous. Thus, the substance appears either to be present in relatively large amounts or to exert its effect in very small quantities. Its mode of action is obscure. Should a loose association or complex with AMP be formed, it must be presumed that this is broken up on elution from the first resin column, since the AMP and ADP are then easily separable on a second column. Nevertheless, the AMP still does not behave quite normally; although it now comes away from the column as a single clear peak with the AMP eluant, more elution than normal is required to release it. It seems probable that a much smaller amount of the interfering substance must be applied to the second column than to the first. On the complexing hypothesis, if there were only sufficient of it to involve a part of the AMP present on this second column, it might be expected that the remaining free AMP would come off in its normal position, but the complexed fraction again only with the stronger

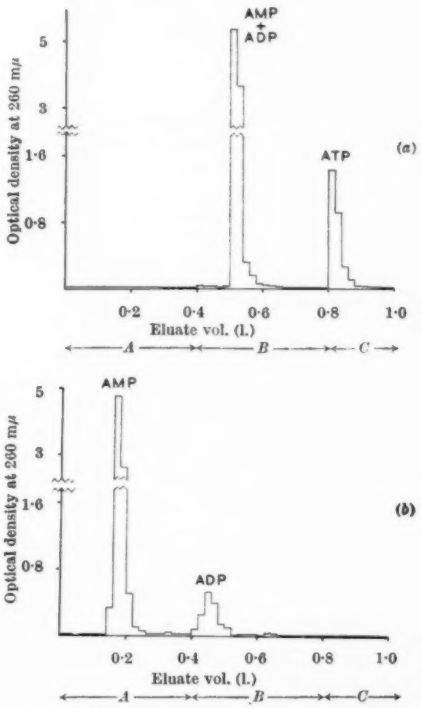


Fig. 2. Passage of a charcoal-treated nucleotide mixture through two successive resin columns. (*a*) Chromatography on first column; (*b*) resolution of the mixed AMP–ADP peak on the second column. Experimental details as in Fig. 1

eluant, so that two peaks should result. Actually there is only one peak, yet a delayed one. Hence on the columns, the AMP always behaves like a single substance the elution point of which is delayed progressively as the amount of the interfering substance increases. No similar interference after charcoal treatment was found when formate columns were used, even though considerably more charcoal was then necessary in the nucleotide isolation step.

Certain anomalies in the chromatographic behaviour of nucleotides resulting from their previous treatment have already been recorded. Lund *et al.*[3] found that treatment of ATP with cation-exchange resins caused it to behave aberrantly when it was chromatographed later on paper, occupying, with some solvents, the position usual for ADP. Laws and Stickland[4] observed that if nucleotides are isolated from yeast by precipitation with barium acetate, the presence of large amounts of water-soluble barium salts causes AMP to behave abnormally during chromatography; on the anion-exchanger 'Amberlite *CG*–400' it is then eluted in a solution intermediate in strength between those usual for AMP and ADP.

This work formed part of a Ph.D. thesis submitted to the University of London; it is a pleasure to acknowledge the guidance and helpful criticism of Dr. F. J. Richards.

U. Pethiyagoda

Agricultural Research Council
Unit of Plant Morphogenesis and Nutrition,
Wye College,
Nr. Ashford, Kent.

[1] Cohn, W. E., and Carter, C. E., *J. Amer. Chem. Soc.*, **72**, 4273 (1950).
[2] Bergkvist, R., and Deutsch, A., *Acta Chem. Scand.*, **8**, 1877 (1954).
[3] Lund, N. A., Grylls, F. S. M., and Harrison, J. S., *Nature*, **173**, 544 (1954).
[4] Laws, J. O., and Stickland, L. H., *Nature*, **184**, 1246 (1959).

Changes in the Concentration of Ribonucleic Acid during Wound Tissue Regeneration

The metabolism of ribonucleic acid (RNA) in regenerating wound tissue may be expected to be of special importance since the synthesis of protein is one of the principal metabolic activities of this tissue[1,2]. The relative amount of RNA in this tissue at different stages of regeneration should give some insight into the protein metabolism. A complicating factor is the fact that considerable amounts of two quite different types of protein are formed, the intracellular 'metabolically-active' proteins, and collagen.

The concentration of RNA in regenerating wound tissue was determined by analysis of tissue obtained from adult female rats. Because of the marked influence of dietary protein on the rate of regeneration[3,4], these animals were fed identical amounts (8 gm./day) of a protein-free diet for 3 days prior to wounding and for the balance of the experimental period. In this way, the experimental animals served as their own source of nitrogen for the synthesis of protein and nucleic acids. The procedure for making standard wounds has been described previously[2,3]. At intervals after wounding, the regenerating tissue was collected and immediately homogenized by repeated grinding in a mortar with sand and small amounts of cold water. After determining the nitrogen in an aliquot of the homogenized tissue (micro-Kjeldahl), the remainder was fractionated to separate the RNA from the deoxyribonucleic acid (DNA) by the method of Schmidt and Thannhauser[5,6]. The phosphate content of the RNA was measured[7]. Less than 3 per cent of the total phosphate in this fraction was inorganic; this inorganic phosphate may have arisen from the hydrolysis of phospho-proteins during the fractionation procedure[8]. Since phospho-lipids, free nucleotides and inorganic and sugar phosphates had been removed by previous procedures, it was assumed that essentially all the phosphate in this fraction must come from RNA.

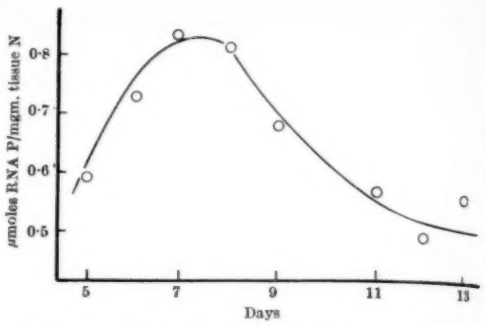


Fig. 1. RNA content of regenerating wound tissue in μmoles of phosphorus per mgm. of tissue nitrogen at various times after wounding. Each point represents the data from the analysis of tissue from 5–6 animals

The level of RNA in the regenerating tissue at various intervals after wounding is shown in Fig. 1. The highest concentration appears to be reached in about 7–9 days after wounding; thereafter it decreases to a lower and relatively constant level. In part, this decrease is due to the accretion of nitrogen in the wound tissue with time. However, even when calculated on the basis of tissue weight, there is still an appreciable decrease in the amount of RNA.

If the types of RNA molecules being synthesized by cells can be shown to change, it might be considered to be indirect evidence that new or different proteins are being synthesized. A change in the type of RNA, or the proportion of the various kinds of RNA produced by the cell, may be signalled by a change in the proportion of purines to pyrimidines of the RNA fraction of the tissue. In a further experiment, it was possible to obtain a sufficient quantity of regenerating tissue to enable both the ribose and phosphate content of the RNA fraction to be measured. The amount of ribose in the RNA fraction was determined by the orcinol method[9]. From the data on the analysis of ribose, which is a measure of the purine nucleotides only[10], and that of the phosphate, which is proportional to the total nucleotide content of the nucleic acid, a ratio which approximates the proportion of pyrimidines to purines in the RNA may be calculated (Table 1). During the early phases of regeneration, the RNA in the wound tissue contains about the same amounts of purine and pyrimidine nucleotides, on a molar basis; later the RNA fraction

Table 1. Purines and Pyrimidines in RNA of Regenerating Wound Tissue

Days after wounding	μmoles Ribose per mgm. tissue nitrogen	μmoles Phosphate per mgm. tissue nitrogen	Ratio of purine to pyrimidines*
5	0·25 ± 0·08	0·46 ± 0·11	1·11 ± 0·12
8	0·60 ± 0·11	1·16 ± 0·12	1·05 ± 0·07
12	0·41 ± 0·09	1·01 ± 0·11	0·70 ± 0·07
15	0·33 ± 0·04	0·88 ± 0·12	0·61 ± 0·09

* The standard error was calculated from the purine/pyrimidine ratios for individual tissue samples.

appears to contain an appreciably larger proportion of pyrimidine than purine nucleotides.

This change in proportion of nitrogen bases might be interpreted to mean that different types of RNA are being synthesized at different stages of regeneration; this, in turn, may presage a change in the nature of the protein synthesized in the regenerating tissue. Large amounts of collagen begin to appear in the wound tissue only after the 8th day after wounding[11,12]. Since the amino-acid composition of collagen (and presumably its intracellular precursors also) is so radically different from that of the 'metabolically-active' proteins, it does not seem improbable that a type of RNA with radically different nucleotide composition might be involved in the synthesis of the collagen precursors. It must not be overlooked that even though much collagen may be formed, some of the 'metabolically-active' proteins are also being formed at the same time. Thus, if the change in the nucleotide composition is related to the synthesis of collagen precursor, it may be expected that the actual difference in the types of RNA involved is much greater than is indicated by the above results.

This investigation was supported by a research grant from the U.S. Public Health Service.

MARTIN B. WILLIAMSON
WILHELM GUSCHLBAUER*

Department of Biochemistry,
School of Medicine and Graduate School,
Loyola University, Chicago 12, Illinois.

* Fulbright Fellow.

[1] Williamson, M. B., *Clin. Chem.*, **2**, 1 (1956).
[2] Williamson, M. B., and Fromm, H. J., *J. Biol. Chem.*, **212**, 705 (1955).
[3] Williamson, M. B., and Fromm, H. J., *Proc. Soc. Exp. Biol. Med.*, **83**, 329 (1953).
[4] Cuthbertson, D. P., in *The Biochemical Response to Injury*, edit. by Stoner, H. B., and Threfall, C. J., 193 (C. C. Thomas, Springfield, Ill., 1960).
[5] Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, **161**, 83 (1945).
[6] Schneider, W. C., *J. Biol. Chem.*, **164**, 747 (1946).
[7] Leloir, L. F., and Cardini, C. E., in *Methods in Enzymology*, edit. by Colowick, S. P., and Kaplan, S. D., **3**, 840 (Academic Press, 1957).
[8] Davidson, J. N., Frazer, S. C., and Hutchison, W. C., *Biochem. J.*, **52**, 594 (1952).
[9] Mejbaum, W., *Z. physiol. Chem.*, **258**, 117 (1939).
[10] Volkin, E., and Cohn, W. E., in *Methods of Biochemical Analysis*, edit. by Glick, D., **1**, 287 (Academic Press, 1954).
[11] Dunphy, J. E., and Udupa, K. N., *New England J. Med.*, **253**, 847 (1955).
[12] Williamson, M. B., and Guschlbauer, W. (unpublished results).

Phosphorylserine as a Constituent of Phosphoproteins from Plasma of the Laying Hen

ALTHOUGH it has been well established that the blood of the laying hen contains phosphorus compounds, the quantities of which are increased immediately preceding and during ovulation[1,2], the nature of some of these substances has only recently been subject to chemical investigation. Dilution of plasma from the laying hen results in the precipitation of a complex of lipid and a phosphoprotein, though by far the greater proportion of the total phosphoprotein present in the plasma remains in the supernatant[3]. Designation of these materials as phosphoproteins has rested entirely on the finding that inorganic phosphate is liberated when they are incubated with dilute alkali[3], but the manner in which the phosphorus is bound has not been reported. Phosphoproteins in general and particularly that from egg yolk, contain their phosphorus as phosphorylserine[4,5], and this has now been sought in acid hydrolysates of both the phosphoprotein fractions of the plasma.

Plasma from laying hens was diluted with 9 vol. water[2] and the precipitated lipophosphoprotein collected by centrifugation. The supernatant was precipitated by trichloroacetic acid added to a final concentration of 10 per cent (w/v) and both this precipitate and that of the lipophosphoprotein was extracted with chloroform/methanol (2 : 1 v/v)[6]. The lipid-free residues were hydrolysed in 2 *N* hydrochloric acid at 100° and the supernatants, containing 80–96 per cent of the total phosphorus of the fractions, were dried over phosphorus pentoxide and potassium hydroxide. The residues were fractionated on 'Dowex 50' (× 12)[7]. After elution of a fraction containing inorganic phosphate a second major phosphate fraction was eluted at a point corresponding to that at which phosphorylserine was eluted when run through the same column. No other fractions were detected. The presumptive phosphorylserine fraction contained between 20 and 54 per cent of the organic phosphorus present in the hydrolysate added to the column and was further purified by high-voltage electrophoresis on paper at *p*H 1·5 (ref. 8). Under these conditions 80–86 per cent of the phosphorus moved as a single component at a rate identical with phosphorylserine. The remaining 14–20 per cent formed a single slower-moving component. Both components reacted with ninhydrin. When eluted and hydrolysed to yield the constituent amino-acids, two-dimensional paper chromatography showed that the component migrating at the same rate as phosphoserine yielded only serine. The molar ratios of phosphorus to serine in two experiments were 1·0 and 1·3. The second minor fraction yielded mainly serine and glutamic acid with traces of alanine and possibly leucine. The total yield of phosphorylserine was 38–39 per cent of the organic phosphorus of the phospholipoprotein residues and 17–20 per cent of that present in hydrolysates of the phosphoprotein derived from the supernatant. A specimen of phosvitin subjected to the same procedure yielded phosphorylserine equivalent to 25 per cent of the organic phosphate present in the hydrolysate. No phosphoproteins were detected in plasma of the non-laying hen or the cockerel (cf. ref. 2).

These experiments appear clearly to establish phosphorylserine as being the only major single phosphorylamino-acid detectable in partial hydrolysates of phosphoproteins from plasma of the laying hen. The overall recovery, when compared with that obtained with phosvitin, suggests that all the phosphoprotein phosphorus may be bound in this manner.

I thank Dr. M. E. Coates, National Institute of Research for Dairying, Shinfield, Nr. Reading, for a generous supply of birds.

P. J. HEALD

Twyford Laboratories, Limited,
Twyford Abbey Road,
London, N.W.10.

[1] Laskowski, M., *Biochem. Z.*, **275**, 293; **278**, 345 (1935).
[2] McIndoe, W. M., *Biochem. J.*, **72**, 153 (1959).
[3] Plimmer, R. H. A., and Bayliss, W. M., *J. Physiol.*, **33**, 439 (1906).
[4] Lipmann, F., and Levene, P. A., *J. Biol. Chem.*, **98**, 109 (1932).
[5] Mecham, D. K., and Olcott, H. S., *J. Amer. Chem. Soc.*, **71**, 3670 (1949).
[6] Folch, J., Ascoli, I., Lees, M., Meath, J. A., and LeBaron, F. N., *J. Biol. Chem.*, **193**, 833 (1951).
[7] Kennedy, E., and Smith, S. W., *J. Biol. Chem.*, **207**, 153 (1954).
[8] Williams, J., and Sanger, F., *Biochim. Biophys. Acta*, **33**, 294 (1959).

Distribution of Ribonucleic Acid in Subcellular Fractions of Various Regions of the Central Nervous System of the Rat

The distribution of ribonucleic acid (RNA) in the subcellular structures of whole brain was studied by Brody and Bain[1], and by Aldridge and Johnson[2], and in the rat brain cortex during development by Keup[3]. We showed previously that appreciable differences exist in the distribution of nucleotides and RNA and in the incorporation of phosphorus-32 in this RNA in various brain regions[4]: we have now studied the RNA content of the subcellular fractions of each region of the central nervous system examined previously and the incorporation of phosphorus-32 in the RNA fractions thus isolated.

We have completed six experiments using twenty rat brains on each occasion. The rats received intraperitoneal injections of phosphorus-32 (200 μc./100 gm. body-weight) and were decapitated after one, three and five days. The heads were immediately plunged into ice-cold 0·9 per cent sodium chloride and the brains were removed rapidly in a manipulation cabinet at 0° C. Various parts of the brains were separated by dissection: olfactory lobes, grey matter and white matter of the cerebrum, hypothalamus, cerebellum and medulla oblongata. The subcellular fractions were isolated using 0·3 *M* sucrose by the classical method of Hogeboom, Schneider and Pallade[5], as slightly modified by Aldridge and Johnson[2]. The purification of the nuclei did not seem sufficient and we therefore employed the following modification. Centrifuging at 1,000*g* for 10 min. with a 0·3 *M* sucrose solution gave a precipitate mixed with capillaries and blood, these forming a lower layer in the centrifuge tubes. The upper layer was separated, re-homogenized with 0·3 *M* sucrose and centrifuged at 1,000*g* for 10 min. The precipitate obtained was mixed with 20 volumes of 1·5 *M* sucrose and centrifuged at 145,000*g* for 90 min. in a 'Spinco' model *L* ultracentrifuge. This treatment gave a precipitate consisting of nearly pure nuclei and a supernatant film containing myelinated structures slightly contaminated with their cytoplasm and with axonal fragments. These facts were demonstrated by electron microscopy. It was estimated that about 90 per cent of the supernatant consisted of myelinated structures. This was confirmed by the chemical analysis, which showed that the supernatant contained, on average, about 55 per cent of the total phosphatides for all the different parts, while the cytoplasmic fluid contained only about 2 per cent and the totality of mitochondria 24 per cent[6].

The heavy and light mitochondrial fractions and the microsomes were separated by the method of Aldridge and Johnson[2].

After extraction of the acid-soluble matter by 0·6 *N* perchloric acid, each subcellular fraction was treated with cold ethanol and then, to extract the lipids, treated by the method of Bloor[7]. RNA and deoxyribonucleic acid (DNA) were separated by the procedure of Schmidt and Thannhauser[8] and the RNA fraction was purified by a technique described elsewhere[4]. The specific activity of RNA phosphorus was determined on solutions at infinite thickness in an end-window Geiger counter (mica window 1·8 mgm./cm.^{2}). The results of the experiments are shown in Tables 1 and 2.

The ratio of RNA to protein was found to be lowest (0·29–0·57) in the myelin fraction, higher in the heavy mitochondria (0·54–1·08) and, on average, higher still in the light mitochondria (1·01–1·4), while for the nuclei the values varied from 0·98 to 1·69. For the cytoplasmic fluid the values were of the same order, although rather lower in the cerebellum and the medulla. The highest values were, of course, found in the microsomes. For a given subcellular fraction clear and important differences existed between one part of the brain and another: the highest values for the nuclei correspond to hypothalamus, followed by the olfactory lobes and the grey matter of the cerebrum; the lowest values were for the cerebral white matter and the medulla. The RNA content of the microsomes was fairly constant at about 30 per cent of the total for each region while for the other fraction the RNA content varied appreciably from one region to another. The ratio RNA/DNA, which also gives an indication of the purity of the nuclei, varied between 0·08 and 0·17: the highest values were for the hypothalamus and the cerebral grey matter.

The specific activity of the nuclear RNA at one and three days is clearly higher than that of the other fractions. The same is true at 5 days except for the olfactory lobes and the hypothalamus. A comparison of the results for the specific activity with respect to

Table 1. Distribution of Ribonucleic Acid in Subcellular Fractions of Various Regions of the Rat Central Nervous System

Region	Nuclei		RNA/DNA	Heavy mitochondria		Light mitochondria		Endoplasmic reticulum (microsomes)		Cytoplasmic fluid		Myelin fraction	
	μgm. P/mgm. protein	Percentage of total RNA		μgm. P/mgm. protein	Percentage of total RNA	μgm. P/mgm. protein	Percentage of total RNA	μgm. P/mgm. protein	Percentage of total RNA	μgm. P/mgm. protein	Percentage of total RNA	μgm. P/mgm. protein	Percentage of total RNA
Olfactory lobes	1·27	14·8	0·105	0·99	6·8	1·40	6·9	3·43	30·0	1·35	24·0	0·55	17·4
Cerebral grey matter	1·23	9·0	0·166	0·54	9·3	1·26	8·1	3·55	33·9	1·44	24·0	0·35	15·8
Cerebral white matter	1·05	10·3	0·124	0·55	9·7	1·00	7·3	2·35	29·5	1·00	21·8	0·34	21·0
Hypothalamus	1·69	10·0	0·178	0·56	12·1	1·01	10·1	2·27	32·6	1·06	20·0	0·57	15·0
Cerebellum	1·20	32·4	0·081	1·08	5·3	1·06	5·2	3·49	31·0	0·65	10·6	0·49	15·4
Medulla oblongata	0·98	9·3	0·154	1·01	16·1	1·21	10·9	2·45	31·6	0·68	14·9	0·29	17·2

Table 2. Specific Activity in Counts/Min. μgm. of Ribonucleic Acid Phosphorus in Subcellular Structure of Various Regions of the Rat Central Nervous System

Region	Hr.	Nuclei	Heavy mitochondria	Light mitochondria	Endoplasmic reticulum (microsomes)	Cytoplasmic fluid	Myelin fraction
Olfactory lobes	24	37	24	10	20	33	36
	84	75	39	40	51	57	49
	120	52	—	—	68	69	54
Cerebral grey matter	24	34	9	16	9	13	24
	84	43	29	25	23	30	24
	120	57	32	30	40	41	27
Cerebral white matter	24	26	7	8	8	13	17
	84	46	26	21	27	32	29
	120	49	30	28	37	39	35
Hypothalamus	24	58	24	11	30	19	34
	84	64	36	38	44	47	47
	120	51	51	42	51	88	29
Cerebellum	24	39	9	9	14	16	—
	84	46	34	31	39	55	47
	120	57	—	—	46	64	46
Medulla oblongata	24	50	9	18	15	13	28
	84	56	30	38	36	38	45
	120	71	—	—	53	39	40

time for the nuclear RNA shows that a maximum exists between the first and fifth day for the olfactory lobes and the hypothalamus, while for the other regions the specific activity at five days is either greater than or equal to that at three days. For the hypothalamus and the olfactory lobes the specific activity versus time curve for the nuclei crosses those for the cytoplasmic fluid and the microsomes. Thus, our results confirm previous data concerning the high RNA content and great metabolic activity of the olfactory lobes and the hypothalamus[4].

The relatively high RNA content of the myelin fraction recalls former investigations concerning the cytoplasm attached to myelin sheaths and the RNA content of axons. It should be mentioned that the relatively high specific activity of the RNA of the myelin fraction cannot be entirely accounted for by a contamination of nuclei, since 90 per cent of the nuclei of the various regions, apart from the cerebellum, contains, on average, only about 10 per cent of the total RNA. In addition, 24 hr. after injection the myelin fraction has a specific activity superior to that of any other subcellular fraction apart from the nuclei. It is therefore difficult to attribute this activity to a contamination by mitochondria, microsomes or cytoplasmic fluid.

Our nuclear fraction contained 70 per cent of the total DNA in the cases of the cerebral white matter and medulla; 75, 86, 89 and 92 per cent respectively for the hypothalamus, cerebral grey matter, olfactory lobes and the cerebellum. It should be noted that the ratio RNA/DNA for our nuclear fraction is generally lower than or at the most equal to that found by Chauveau[9] for the liver and is, for three regions, near that found by Georgiev and Mantiewa[10] for the whole brain.

T. Borkowski

Zaklad Chemii Fizjologicznej Akademii,
Medycznej, Lublin.

S. Harth
R. Mardell
P. Mandel

Institut de Chimie Biologique,
Faculté de Médecine,
Strasbourg.

[1] Brody, T. M., and Bain, J. A., *J. Biol. Chem.*, **195**, 685 (1952).
[2] Aldridge, W. N., and Johnson, M. K., *Biochem. J.*, **73**, 270 (1959).
[3] Keup, W., *Ultrastructure and Cellular Chemistry of Neural Tissue*, edit. by Waelsch, H., 215 (Hoeber-Harper, New York, 1957).
[4] Mandel, P., Harth, S., and Borkowski, T., *Fourth Intern. Symp. Neurochem., Varenna*, 1960 (Pergamon Press, Ltd., 1961).
[5] Hogeboom, G. H., Schneider, W. C., and Pallade, C. E., *J. Biol. Chem.*, **172**, 619 (1948).
[6] Harth, S., Borkowski, T., Mardell, R., and Mandel, P. (in the press).
[7] Bloor, E., *J. Biol. Chem.*, **82**, 273 (1929).
[8] Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, **161**, 83 (1945).
[9] Chauveau, J., Moule, I., and Rouiller, Ch., *Bull. Soc. Chim. Biol.*, **39**, 1521 (1957).
[10] Georgiev, G. P., and Mantiewa, V. L., *Biochimia*, **25**, 143 (1960).

Cystine Content in Hair of Childen as influenced by Vitamin A and Animal Protein in the Diet

Recently, Koyanagi and Odagiri[1] reported that the cystine content of skin of rats dosed with a small quantity of vitamin A is lower than that of rats given a liberal allowance of the vitamin. In the presence of methionine, the cystine content increased both with small and large doses of vitamin A. We now wish to report on experiments conducted to determine whether the cystine content in the hair of children could be changed by deficiencies of vitamin A in the diet and other factors.

Table 1. Amount of Foods Consumed and Estimate of Protein and Vitamins per Person per Day

Foods	District *A* (gm.)	District *B* (gm.)	District *C* (gm.)
Rice	—	492	300
Barley	150	9	60
Wheat	100	21	100
Japanese barnyard millet	400	—	—
Potatoes	100	22	50
Sugar	—	3	20
Oils and fats	—	6	8
Soybean	54	4	5
Miso (fermented soybean)	100	49	30
Fish, raw	20	100	100
Fish, salted	—	10	10
Tofu (soybean curd)	—	34	80
Meat	—	4	30
Eggs	—	18	30
Milk	—	14	100
Vegetable, green	50	43	100
Vegetable, uncoloured	100	64	100
Vegetable, salted	200	100	70
Fruits	—	9	150
Calories	2,846	2,216	2,549
Protein* (gm.)	119·0 (3·8)†	74·6 (27·6)	60·4 (38·5)
Vitamin A (I.U.)	1,184	1,235	3,572
Riboflavin (mgm.)	0·71	0·63	1·40
Thiamine (mgm.)	1·97	0·92	1·21

* Digestibility of protein of barnyard millet is very low.
† Amount of animal origin indicated in parentheses.

The survey was done on school children aged 8–9 yr., in the following three districts; (*A*) Yamagata village, situated in the mountainous area where people having a small income eat Japanese barnyard millet and very little fish; (*B*) Teshiromori village, situated in the rice field district where people live on rice and eat a moderate amount of fish; school lunches are served; (*C*) Morioka city, where the diets are comparatively well balanced; children have lunch served at school. Food articles available for civilian consumption in three districts are shown in Table 1.

To determine the cystine content in the hair, 0·1 gm. of hair from the back of the head was hydrolysed in 3 *N* hydrochloric acid at 120° C. for 4 hr. The cystine content in the hydrolysate was analysed by the phosphotungstic acid method[2].

Effect of diet. The results (Table 2) indicate that there are striking differences in cystine content in the hair from the three districts; for example, the figures from district *A* show only 8·1 per cent while those from districts *B* and *C* return 11·4 and 12·5 per cent, respectively. The figures thus correlate closely with the consumption of animal protein.

Effect of vitamin A and milk. Table 2 gives results in districts *A* and *B* obtained by analysing hair of children supplemented for half a year with shark liver oil (2,500 I.U.). The cystine content is higher in the hair of children in the supplemented group. The observations are in agreement with those on the skin of rats reported by Koyanagi *et al.* In district *B* some children were allowed to drink milk liberally, that is, 300–400 c.c. daily a child for half a year. The cystine content of their hair is remarkably higher than that of children without milk.

Table 2. Cystine Content in the Hair of Children in Three Districts

District	Supplement	Height (cm.)	Weight (kgm.)	Cystine in the hair (per cent)	
A	None	116·3 ± 6·7*	21·9 ± 2·2*	8·1 ± 0·15*	(21)†
	Shark liver oil	122·0 ± 5·2	22·0 ± 2·2	9·5 ± 2·3	(11)
B	None	121·0 ± 3·1	24·1 ± 1·7	11·4 ± 1·8	(13)
	Shark liver oil	124·7 ± 4·5	24·5 ± 2·6	13·0 ± 1·4	(12)
	Milk	128·7 ± 3·1	26·2 ± 0·5	14·4 ± 0·6	(9)
C		127·5 ± 1·8	26·7 ± 1·2	12·5 ± 1·3	(22)

* Standard deviation of the mean.
† No. of children indicated in parentheses.

Effect of age. Hair of 26 infants was collected in district *C* and analysed. In this case, in spite of individual variation the higher values were found more often among the younger group and there was no apparent difference between sexes. (9 months, 12·1–15·1; 3 yr., 11·9–14·4; 4 yr., 10·6–13·0; 5 yr., 10·5–13·0; 6 yr., 11·0–12·7; adults, 10–12 per cent.)

Effect of disease. Through the clinical examination conducted on 75 infants in district *A*, 13 cases of active angular stomatitis, 25 cases of fatty liver and 5 cases of severe malnutrition were found. It is remarkable that the cystine content in the hair of infants showing undernutrition showed strikingly lower values than in that of the healthy infants in district *C*. (2 yr., 6·0–9·2; 3 yr., 5·5–6·6; 4 yr., 5·8–9·2; 5 yr., 7·2 per cent.)

In regard to amino-acid content of nails, Hess[3] reported that the molecular ratios of histidine to lysine to arginine for normal nails were 1 : 6 : 13 while for arthritic nails they were 1 : 5·4 : 13·5. The three basic amino-acids remained practically constant, while the cystine content decreasd 18·4 per cent. So it is fair to state that the cystine content in the hair may be influenced by the condition of health, and by disease, as well as through the diet. Observation on the cystine content of hair led to a postulation that the cystine content of hair may be looked on as a sign of nutritional status for children, or as an indication of the presence of some disease.

Tatsuo Koyanagi
Department of Agricultural Chemistry,
Teru Takanohashi
Department of Home Economics,
Iwate University, Morioka, Japan.

[1] Koyanagi, T., and Odagiri, S., *Nature*, **186**, 809 (1960).
[2] Kassel, B., and Brand, E., *J. Biol. Chem.*, **125**, 115 (1938). Kolb, J. J., and Toennies, G., *Anal. Chem.*, **24**, 1164 (1952).
[3] Hess, W. C., *J. Biol. Chem.*, **109**, 43 (1935).

PHYSIOLOGY

Amounts of Oxides of Nitrogen and Carbon Monoxide in Cigarette Smoke, with and without Inhalation

Recently, Haagen-Smit[1] has referred to the relatively high content of oxides of nitrogen in smoke from cigarettes and cigars. It was found that the content of oxides of nitrogen in cigarette smoke varies from 145 to 655 p.p.m. Furthermore, after inhalation of the smoke into the lungs, no oxides of nitrogen could be detected in the exhaled smoke. In view of the very low maximum concentrations of oxides of nitrogen tolerated (Russian workers[2] claim 0·1 mgm./m.³ as an average concentration during 24 hr., and 0·3 mgm./m.³ as a maximum concentration at one time; for carbon monoxide these concentrations are 2 and 6 mgm./m.³ respectively) it was thought interesting to determine how much of these compounds may be taken up as a result of cigarette smoking.

Two types of experiments were carried out. A smoking apparatus was developed in which normal smoking could be simulated. It consists essentially of a spiral washer with xylene for the removal of tars, which may affect the reaction with the Griess-Ilosvay reagent, and of a sampling bottle, a gas meter and finally a magnetically operated valve. The latter is connected, through a capillary, with the vacuum line. Smoke from a cigarette was drawn in 27-ml. samples into the sampling bottle by opening and closing (2 and 28 sec. respectively) the magnetically operated valve. The natural smoking process was simulated to such a degree that 15 × 0·5 = 7·5 min. were required to smoke a cigarette to a residual length of about 10 mm. During this time about 400 ml. gas was delivered into the sampling bottle.

In each sample the contents of nitrogen oxides and carbon monoxide were determined by normal analytical methods. In a few cases a distinction was made between nitric oxide and nitrogen dioxide. From Table 1 it appears that under our smoking conditions approximately equal amounts of nitric oxide and nitrogen dioxide are present. The total amount of nitrogen oxides is within the range found by Haagen-Smit for different types of cigarettes.

Table 1. Concentrations of Oxides of Nitrogen and Carbon Monoxide in Cigarette Smoke

(King-size cigarette, relative humidity 45 per cent)

Cigarette	$NO + NO_2$ (p.p.m.)	NO_2 (p.p.m.)	CO (per cent by volume)	CO_2 (per cent by volume)
1	190			
2	170			
3	180			
4	210			
5	210			
6	180			
7	170			
8	190			
9	190	100	2·2	7·8
10	200	120	2·0	7·0
11	190	80	2·1	

Inhalation experiments were carried out by three separate persons. In each experiment a cigarette was smoked in such a way that 500 ml. smoke + additional air were inhaled during each inhalation. After exhalation into a 10-litre sampling bottle, the lungs were flushed with four times the amount of air, which was also passed into the sampling bottle. In addition, non-inhalation experiments were carried out by the same persons. The smoke of each pull was kept in the mouth, and then passed into the sampling bottle where it was diluted with air (inhaled through the nose) to a total volume of 2·5 l. In both cases 13 × 2·5 = 32·5 l. were passed through the bottle, after which the amounts of nitrogen oxides and carbon monoxide were determined.

The figures given in Table 2 show that, as a result of inhalation, both the nitrogen oxides and the carbon monoxide are absorbed to a large extent. Consequently the amount of these compounds which may be taken up by the body can readily be estimated. If 20 cigarettes a day are smoked, 3·0 mgm. of oxides of nitrogen (calculated as nitrogen dioxide) and 200 mgm. of carbon monoxide may be absorbed. These figures are considerably higher than the amounts,

Table 2. Concentrations of Oxides of Nitrogen and of Carbon Monoxide in Cigarette Smoke, with and without Inhalation

Person	CO (per cent by volume) Without inhalation	CO (per cent by volume) With inhalation	Absorbed per cent	Oxides of nitrogen ($NO + NO_2$) (p.p.m.) Without inhalation	Oxides of nitrogen ($NO + NO_2$) (p.p.m.) With inhalation	Absorbed per cent
A	2·1*	0·37	82	180	23	87
B	2·1*	0·36	82	240	11	95
B				220	12	95
C	2·1*	0·27	87	180	8	96
C		0·37	82	220	10	96

*The concentrations have been converted to those in the undiluted smoke. The dilution factor was calculated from the content of carbon monoxide in the 10-l. sampling bottle and the average value of 2·1 per cent as determined from the experiments with the smoking apparatus.

namely $12 \times 0{\cdot}1 = 1{\cdot}2$ mgm. oxides of nitrogen and $12 \times 2 = 24$ mgm. of carbon monoxide, regarded as the maximum which can be safely tolerated.

Whether the effect of incidental inhalation of relatively high concentrations of noxious compounds is greater than that from continuous inhalation of a much lower concentration is difficult to establish. It should be remarked, however, that for an evaluation of the health effects of smoking the possibility of synergism[3], caused by the simultaneous presence of both nitrogen oxides and carbon monoxide, should also be taken into account.

C. Bokhoven
H. J. Niessen

Central Laboratory,
Staatsmijnen in Limburg,
Geleen, The Netherlands.

[1] Haagen-Smit, A. J., Brunelle, M. F., and Hara, J., *Arch. Indust. Health*, 20, 399 (1959).

[2] Riasanov, "New Data on Maximum Allowable Concentrations of Pollutants in the Air in the U.S.S.R." (International Clean Air Conference, London, 1959).

[3] von Oettingen, W. F., *Pub. Health Bull.* No. 290, U.S. Government Printing Office, 1944. Stokinger, H. E., *Int. J. Air Poll.*, 2, 313 (1960).

Sodium and Potassium Content in the Muscles of Hibernating Animals

It has long been known that tissues exposed to low temperatures *in vitro* lose potassium while on the other hand there are few published reports regarding sodium exchange during the same conditions[1,2]. In a previous report[3] we have demonstrated that the potassium/sodium ratio in the muscles of fishes caught in cold arctic waters is low when compared with fishes from boreal waters. The very same variations with temperature were found to take place in specimens of the same species both in Nature (*Gadus*) and in the laboratory (*Cottus*).

As we were at the same time working with problems on the physiology of hibernation we were led to suspect that the extreme hypothermia normally occurring during hibernation in these animals might induce even more marked changes in electrolyte composition of the muscle cells.

The animals used were the common European hedgehog, *Erinaceus europaeus* L., and the bat, *Myotis daubentonii* L. The muscle samples were dry-ashed at 550° C. and the content of sodium and potassium estimated by flame-photometry.

In the case of the hibernating animals referred to, we found a rather surprisingly high content of sodium in the muscles, compared with that reported in the literature on other mammals, and also confirmed by my own analysis on the rat, Fig. 1. Furthermore, we found the sodium content to be fairly constant throughout the year, despite the extreme changes in body temperature taking place during this time.

Thus, when compared with other homoiothermic vertebrates having about the same plasma composition, the hibernators differ greatly. Whether or not this has something to do with differences in the physiology of the muscle cells, the present results do not demonstrate. It seems to us, however, that the hibernators, which behave much like poikilotherms in the winter, may somehow be prepared by a high sodium-level to stand the effect of low temperatures on the sodium content of the tissue.

It may be worth considering the same tendency found in arctic fishes, where a high sodium content was encountered at low temperatures. It thus seems

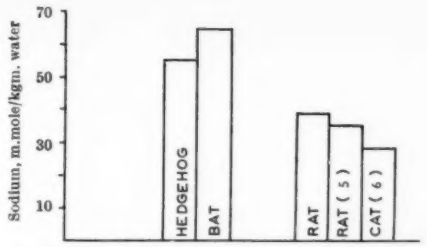


Fig. 1. Sodium content in the muscles of hibernating animals (hedgehog, bat) and some other mammals (refs. 5, 6)

that the ability to stand low temperatures may in some way or other be connected with the sodium metabolism of the cell.

Whereas the level of sodium is fairly constant throughout the year, we found distinct changes in the potassium content of the muscles. Muscle samples have been taken from hedgehogs in the period from May to March, while the samples from bats are from November to April only. As seen in Fig. 2, there is an increase in the potassium content of the hedgehog muscles during the first months of hibernation (October–December) and from then on a distinct decrease. In the months October–November the body temperature of the hedgehog varies greatly, changing from the normal level (35° C.) to environmental temperatures (5° – 8° C. in our animal room) in less than 24 hr. In this 'pre-hibernating' period some animals take food, while others are in inanition, with no apparent effect on the sodium–potassium level. From about December the animals are in deep hibernation. As regards the bats their body temperatures change in about 10–30 min., and their period of inanition begins about September and lasts until about May.

Contrary to the expectations we might have from our results obtained on fish muscles, we thus see that the hedgehog muscles gain potassium in the first part of the hypothermic period. It is rather difficult to explain this considering the temperature effect alone. However, we want to direct attention to certain observations made in this Laboratory[4] on changes in some blood constituents in the 'pre-hibernating' period. In this period we found a marked hæmolysis corresponding to one-third of the total erythrocyte volume. A tentative explanation might therefore be

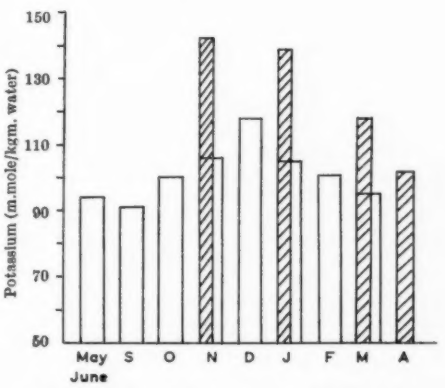


Fig. 2. Potassium content in the muscles of hedgehog and bat at different times of the year. □, hedgehog; ▨, bat

that part of the potassium set free during this hæmolysis is accumulated by the tissue cells. Unfortunately we are at present unable to present enough data on the level of potassium in plasma. Only small changes in this level are necessary, however, to induce an increase in cellular potassium, due to the effect of the Gibbs–Donnan equilibrium. This explanation seems the more reasonable as we found the time of the year to be the important factor: no differences in muscle potassium were observed between samples from animals with normal or hypothermic body temperature.

The effect of hæmolysis in October–November seems to last until December. This lag might be explained on the assumption that the extrusion of salts through the kidney is extremely slow in animals in deep hibernation. With the progress of time (December–March) this slow process, however, leads to a reduction of cellular (and plasma) potassium.

Regarding our results for bats, these are in accordance with such considerations.

Einar Eliassen
Helge Leivestad

Zoological Laboratory,
University of Bergen,
Norway.

[1] Taylor, I. M., *Nat. Acad. Sci. Nat. Res. Council, Washington*, **451**, 26 (1956).
[2] Hashish, S. E. E., *Acta Physiol. Scand.*, **43**, 189 (1958).
[3] Eliassen, E., Leivestad, H., and Möller, D., *Univ. Bergen. Arb. Naturv.*, **14** (1960).
[4] Eliassen, E., *Nature* (in the press).
[5] Conway, E. J., *Biol. Rev.*, **20**, 56 (1945).
[6] Fenn, W. O., and Cobb, D. M., *Amer. J. Physiol.*, **121**, 595 (1958).

Diuresis due to Stress in Cattle

In the course of investigation of some aspects of renal function in adult dairy cattle, it was noted that there was a marked diuretic response to a painful stimulus. The animals used were non-lactating, non-pregnant Ayrshire dairy cows accustomed to experimental procedures, and the stimulus was puncture of the brachial artery by the technique described by Fisher[1], which, despite the use of local anæsthetic, always caused some discomfort. Arterial puncture was carried out during continuous urine collection by an indwelling urethral catheter for renal clearance measurements. In association with the increased rate of flow after arterial puncture, urine became noticeably paler in colour, and there was a marked fall in urinary bicarbonate concentration. Cystometric measurement of bladder pressure showed that there was no variation in intracystic pressure at the onset of diuresis, and, on the basis of these observations, it was assumed that the increase in the rate of urine collection was a true reflexion of increased rate of formation, rather than the expulsion of uncollected urine from the bladder.

The discomfort of arterial puncture, in some animals, caused an immediate diuresis of 200–300 per cent of the previous mean rate of urine flow. The onset and regression of this response were usually rapid. One cow, with a mean rate of urine flow of 9·6 ml./min., showed a diuresis of 28·5 ml./min. in the 5 min. following arterial puncture, the rate returning to 8·5 ml./min. in the next 5 min. period. After repeated experiments on the same animals, this type of response was preceded by a diuresis associated with the preliminary procedure of clipping, swabbing, and anæsthetizing the site for puncture. Fig. 1 illustrates the diuresis which occurs on carrying out

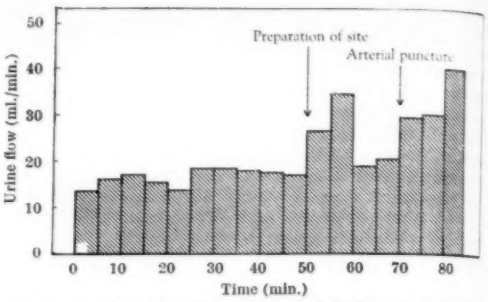


Fig. 1. The effect of arterial puncture, and of clipping, swabbing, and anæsthetizing the site on the urine flow of a normal cow

arterial puncture, preceded by the response to preparation of the site.

Rydin and Verney[2] demonstrated a marked inhibition of urine flow of hydrated dogs following emotional stress, with good evidence that this response was due to the release of antidiuretic hormone. Andersson and Persson[3], however, reported that, in hydrated goats, painful or emotional stimuli did not result in any alteration in the rate of urine formation. In man, Miles and De Wardener[4] described an emotional diuresis which occurred on bladder catheterization, or resulted from apprehension of a surgical procedure. They attributed the rise in salt excretion which accompanied this diuresis to three possible causes: (1) a rise in glomerular filtration-rate; (2) the inhibition of a salt-retaining hormone; (3) a nervous inhibition of tubular function. There are, therefore, marked species differences in renal responses to painful or emotional stimuli.

The mean blood pressure of cows may rise markedly on experimental interference[5], but whether the observations reported here are the result of alterations in renal hæmodynamics or of inhibition of the antidiuretic hormone is not known. It is, however, clear that experiments on cattle which depend on measurement of the rate of urine flow must be carried out with the minimum amount of stress to the subject.

R. S. Anderson

Department of Veterinary Physiology,
University Veterinary School,
Glasgow.

[1] Fisher, E. W., *Vet. Rec.*, **68**, 691 (1956).
[2] Rydin, H., and Verney, E. B., *Quart. J. Exp. Physiol.*, **27**, 343 (1938).
[3] Andersson, B., and Persson, N., *Acta physiol. scand.*, **42**, 257 (1958).
[4] Miles, B. E., and De Wardener, H. E., *Lancet*, ii, 539 (1953).
[5] Doyle, J. T., Patterson, J. L., jr., Warren, J. V., and Detweiler, D. K., *Circulation Res.*, **8**, 4 (1960).

Effects of Thymectomy on the Resistance of Rats to Drowning and Histamine Stress

The thymus has been proposed as a site of hormone production[1]. Bomskov *et al.*[2] have reported that treatment of normal rats and guinea pigs with calf thymus extract distinctly diminished survival-time of animals exposed to chloroform–air mixtures.

The aim of the present work was to determine the degree of resistance conferred by thymectomy on albino male Wistar rats. Two procedures were used, one involving resistance to drowning stress and the second resistance to histamine stress.

Two hundred and forty male rats averaging 50 gm. were matched by weight and divided into three

Table 1. Effect of Thymectomy on Resistance to Histamine and Drowning Stress

Group	n	Initial body-weight gm. ± S.E.	Final body-weight gm. ± S.E.	LD_{50} mgm./kgm. ± S.E.	Drowning time (sec.) ± S.E.	Percentage difference between two groups	P value
1. Histamine stress							
Group *A*: Thymectomy rats	60	52·8 ± 0·4	199·8 ± 2·4	702·9 ± 36·4		A : B = + 6·8 A : C = + 8·2	0·22 0·13
Group *B*: Sham-thymectomy rats	60	52·5 ± 0·5	195·4 ± 2·7	658·4 ± 29·8			
Group *C*: Normal control rats	60	52·5 ± 0·4	200·1 ± 2·3	649·4 ± 33·5		*B* : C = + 1·4	0·76
2. Drowning stress							
Group *A*: Thymectomy rats	19	49·2 ± 0·7	187·6 ± 3·2		541·8 ± 27·8	A : B = + 5·1 A : C = + 8·4	0·51 0·34
Group *B*: Sham-thymectomy rats	19	49·1 ± 0·8	196·8 ± 4·1		515·6 ± 27·3		
Group *C*: Normal control rats	20	49·1 ± 0·5	204·5 ± 3·4		499·7 ± 31·7	B : C = 3·2	0·71

groups, *A*, *B* and *C*, representing respectively thymectomized rats, sham-operated animals and normal controls. The thymectomies were completed under open-ether anæsthesia according to procedures described by Farris and Griffith[3]. At the conclusion of a 4-week period, 60 animals in each group were selected and equal aliquots tested for histamine resistance. The histamine challenge occurred at four dose-levels, 450, 600, 750 and 900 mgm./kgm. of body-weight. Finney's method of probit analysis was used to calculate the *LD*50 values and dose-response lines[4].

The remaining animals were subjected to drowning stress by immersion in water precooled and maintained at 6° C. Drowning time was determined by recording the time-interval up to the final submergence.

Table 1 presents the *LD*50 results. Comparisons between groups *A* and *B* and between *A* and *C* *LD*50 values indicated that thymectomy induced 6·8 per cent and 8·2 per cent respective increases in the tolerance of the thymectomized rats to histamine stress. Although none of these increases was statistically significant, the low P values especially between *A* and *C* approach significance ($P = 0{\cdot}13$).

Table 1 similarly presents the average time of drowning for the three groups of animals immersed in water maintained at 6° C. Again comparisons between groups *A* and *B* and between *A* and *C* indicate respectively 5·1 per cent and 8·4 per cent increases in the resistance of the thymectomized rats to drowning stress. As indicated by the P values, however, none of these increases was statistically significant.

In evaluating the results, it becomes apparent that thymectomy induced similar percentage increases in the resistance of the thymectomized rats to two distinctly diverse types of stress stimuli. The results of this work are thus in accord with previous investigations[2,5]. Although the investigation does not offer conclusive evidence concerning a thymic endocrine function, the results provide support for such a hypothesis.

A. S. Weltman
A. M. Sackler

Laboratories for Therapeutic Research,
Research Laboratories of the Brooklyn
College of Pharmacy,
Long Island University,
Brooklyn, New York.

[1] Bomskov, C., and Spiegel, R., *Endokrinologie*, **23**, 225 (1941).
[2] Bomskov, C., Holscher, B., and Hartmann, J., *Pflüger's Arch. Ges. Physiol.*, **245**, 483 (1942).
[3] Farris, E. J., and Griffith, J. Q., *The Rat in Laboratory Investigation* (J. B. Lippincott Co., Philadelphia, 1949).
[4] Finney, D. J., *Probit Analysis* (Camb. Univ. Press, 1952).
[5] Sackler, A. M., *et al.*, *Fed. Proc.*, **12**, 1196 (1953).

Plasma Iron and Iron-binding Capacity in the Pregnant Rat and Rabbit

In the latter half of human pregnancy a decrease in blood hæmoglobin concentration usually occurs and this is associated with a decrease in plasma iron concentration and an increase in plasma total iron-binding capacity[1,2]. The mechanism producing these changes is not fully understood. They may be due partly to iron deficiency[3,4], one of the main causes of which is the rapidly increasing fœtal utilization of iron. If so, the changes might be more pronounced in rats and rabbits, in which the combined demands of the fœtuses would be relatively greater.

Blood for the various determinations was taken from 7 rabbits weighing 1·8–2·5 kgm. immediately before and 7, 14, 21 and 28 days after mating and on the first day after parturition. Control values were obtained on blood from a comparable group of 8 non-pregnant animals. In these rabbits no significant changes occurred in any of the values measured.

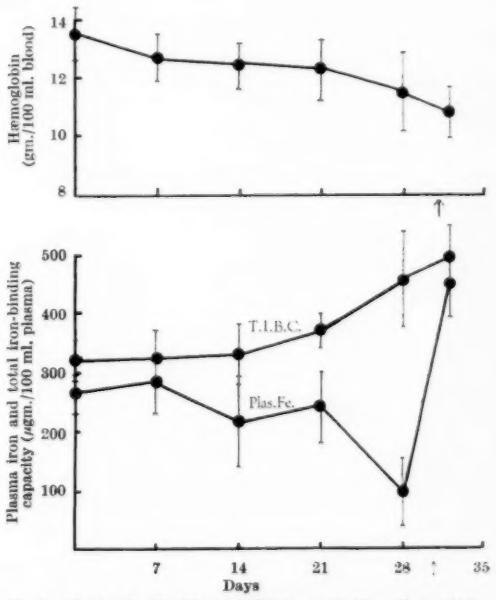


Fig. 1. Changes in blood hæmoglobin concentration, plasma iron concentration (Plas.Fe) and total iron-binding capacity (T.I.B.C.) during pregnancy and after parturition in the rabbit. Each point represents the mean of seven rabbits and the vertical lines represent ∓ one standard deviation of the mean. The time of parturition is shown by the arrow

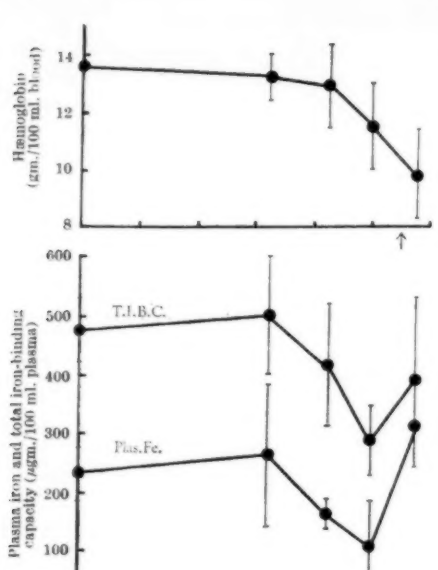


Fig. 2. Changes in blood hæmoglobin concentration, plasma iron concentration (Plas.Fe) and total iron-binding capacity (T.I.B.C.) during pregnancy and after parturition in the rat. Each point represents the mean of six rats and the vertical lines represent ± one standard deviation of the mean. The time of parturition is shown by the arrow

Twenty-four rats aged 18–22 weeks and weighing 190–220 gm. were mated and the day of insemination was determined by daily examination of vaginal smears for spermatozoa. The rats were killed in groups of 6 on day 13, 17 and 20 of pregnancy and on the first day after parturition. A further comparable group of 6 rats was not mated and determinations were made on these to provide non-pregnant control values. The rats were anæsthetized with ether and killed by exsanguination by cardiac puncture.

Blood hæmoglobin concentration[5], and plasma iron and total iron-binding capacity[6], were estimated by methods previously described.

Both rabbits (Fig. 1) and rats (Fig. 2) resembled human beings in showing a decrease in blood hæmoglobin and plasma iron towards the end of pregnancy. Both rabbits and rats, however, showed a considerable increase in plasma iron concentration immediately after parturition, while in humans when such a rise occurs it is small[7]. In contrast to rabbits and humans, rats showed a considerable decrease in plasma iron-binding capacity in the last few days of pregnancy and a marked rise immediately after parturition.

The increase in plasma iron concentration immediately after parturition may be accounted for by the fact that loss of iron to the fœtus is suddenly interrupted. Why plasma iron-binding capacity should fall during pregnancy in the rat and rise during pregnancy in man and the rabbit cannot be explained. An increase in plasma volume occurs during pregnancy in the rat[8], and this could presumably cause some dilution of the iron-binding protein, transferrin. But a similar change in plasma volume also occurs in man[9], where the iron-binding capacity increases during pregnancy[1,2]. In the rat, then, it would seem that the decrease of iron-binding capacity could be better explained by alterations in the rates of synthesis and/or destruction of transferrin or by passage of this protein through the placenta or fœtal membranes.

This work was carried out during tenure of a fellowship of the National Health and Medical Research Council. I am grateful to Miss E. Rudeberg for technical assistance.

E. H. Morgan

Department of Physiology,
University of Western Australia,
Nedlands.

[1] Laurell, C.-B., *Acta Physiol. Scand.*, **14**, Supp., 46 (1947).
[2] Fay, J., Cartwright, G. E., and Wintrobe, M. M., *J. Clin. Invest.*, **28**, 487 (1949).
[3] Sturgeon, P., *Brit. J. Hæmat.*, **5**, 31 (1959).
[4] Morgan, E. H., *Lancet*, i, 9 (1961).
[5] Walsh, R. J., Arnold, B. J., Lancaster, H. O., Coote, M. A., and Cotter, H., *National Health and Medical Research Council (Austral.) Spec. Rep. Series*, No. 5 (1953).
[6] Morgan, E. H., and Carter, M., *Austral. Ann. Med.*, **9**, 209 (1960).
[7] Lunstrom, P., *Acta Soc. Med. Upsaliensis*, **55**, 1 (1950).
[8] Bond, C. F., *Endocrinol.*, **43**, 180 (1948).
[9] Berlin, N., Goltsch, C., Hyde, G., and Parsons, R., *Surg. Gynec. Obstet.*, **97**, 173 (1953).

Importance of Cell Division for Antibody Production in an *in vitro* System

An *in vitro* system for the study of antibody formation has been previously described[1]. In outline, cell suspensions are prepared from rabbit spleens removed two days after anamnestic stimulation with alum-precipitated human serum albumin. The synthesis of antibody *in vitro* is measured by the incorporation of leucine labelled with carbon-14 into antibody protein. This system has been used to determine whether cell division or cell differentiation plays a major part in providing the cell population which synthesizes antibody after anamnestic antigenic stimulation.

The kinetics of antibody synthesis in earlier experiments and the partial inhibition of antibody formation by a thymidine antagonist, 5-bromodeoxyuridine[2], had suggested that 30–80 per cent of the antibody synthesized was made by cells which divided during the 48-hr. incubation period. Autoradiographic studies showed that 2–4 per cent of the incubated cells did indeed take up tritiated thymidine during *in vitro* incubation (to be published), but did not, however, establish that these thymidine-incorporating cells synthesized antibody.

The following experiment was designed to provide more definite proof. Tritiated thymidine of very high specific activity was added to the medium. The dividing cells incorporated, and hence concentrated, the radioactive thymidine into their deoxyribonucleic acid (DNA), which thus resulted in a very selective irradiation of the dividing cells. Under these conditions non-dividing cells would be expected to receive only insignificant irradiation. The addition of tritiated thymidine at 1·5 c./m.mole at the beginning of the incubation period inhibited antibody formation to approximately the same degree as 5-bromodeoxyuridine. Higher specific activities (4·4 c./m.mole) did not result in any appreciably greater inhibition. Dilution of the specific activity of the tritiated thymidine by the additional presence of unlabelled thymidine reduced or prevented the inhibition (see Fig. 1). The inhibition was, therefore, a consequence of an intracellular location of the radioactive thymidine and not simply an unspecific effect of irradiation

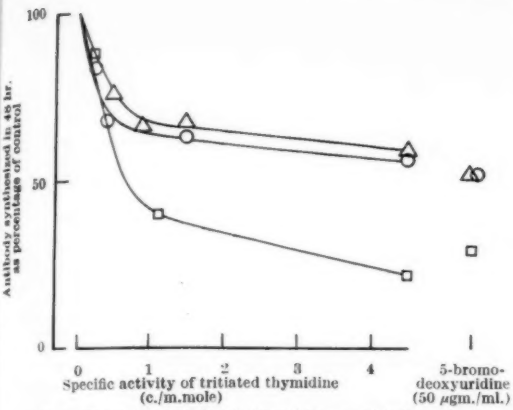


Fig. 1. The effect of tritiated thymidine on antibody synthesis. The three curves—O, Δ and □—are the results obtained with three separate rabbits. The corresponding symbols at the right show the figures for antibody synthesis in the presence of 50 μgm./ml. of 5-bromodeoxyuridine for each rabbit

from radioactivity in the extracellular medium. A further control was provided by experiments using tritiated water. It was found that more than a hundred times as much activity had to be added as tritiated water to produce the same effect as with tritiated thymidine (see Table 1).

That the effect was due to activity concentrated in a most favourable site is supported by a consideration of the calculated doses that the cells received on the basis of various suppositions as to the distribution of the activity (Table 1). By comparison, 200–500 rads of X-irradiation (given at the start of incubation) were required to reduce the antibody synthesis to 50 per cent (to be published). It is not possible to calculate a reliable estimate for the dose received by labelled cell nuclei (see footnote to Table 1) but the figures do provide some basis for comparison.

Table 1. Antibody Synthesis: Comparison of Effect of Irradiation from Tritiated Water and Thymidine

	^{3}H water				^{3}H thymidine
Activity in incubation medium, mc./ml.	59	5·9	0·59	0·059	0·024
Antibody synthesized in 48 hr. as percentage of control	8	37	70	94	42
Dose to cells in rads/hr. assuming uniform distribution throughout medium	710	71	7·1	0·71	0·29
Dose to nuclei, rads/hr. (see footnote)	(a) Assuming synthesis of diploid content of DNA from thymidine at 1·1 c./m.mole				c. 170
	(b) Calculated from observed mean grain count over nuclei after autoradiography				c. 50

In the calculation for the dose-rates to nuclei it has been supposed that the volume of the nucleus was 500 μ^3 and that all the energy was dissipated in this volume. In the calculation from grain counts it has been assumed that the overall efficiency of grain development is 5 per cent. No allowance has been made for the fact that the activity will not be present in the nucleus until the cell has divided. For these and other reasons the figures arrived at can only be taken as a very rough guide to the actual levels of irradiation.

Autoradiographs of smears of the cells which had been incubated with thymidine of high specific activity showed very high levels of activity in the nuclei of 2–3 per cent of the cells. The rest of the cells showed less than a fiftieth of this activity.

Autoradiographs of cells incubated with only tracer-levels (61 mc./m.mole) of tritiated thymidine showed proportionately the same grain count per cell. Thus the high specific activity did not appear to affect the uptake of thymidine into the deoxyribonucleic acid (DNA).

There is, therefore, little doubt that the inhibition of antibody formation was due to irradiation from tritiated thymidine incorporated into the DNA of those cells which were dividing.

These experiments do not show whether or not a cell can divide after it has started to make antibody. In either case it seems likely that most, if not all, of the cells synthesizing antibody have undergone cell division following anamnestic stimulation.

These results are thus in agreement with the recent findings of Urso and Makinodan[3] and Baney, Vazquez and Dixon[4], which were obtained by other techniques.

This work was supported, in part, by U.S. Public Health Service grant *E* 2911.

R. W. Dutton

Department of Chemical Pathology,
Postgraduate Medical School,
Ducane Road, London, W.12.

[1] Vaughan, J. H., Dutton, A. H., Dutton, R. W., George, M., and Marston, R. Q., *J. Immunol.*, **84**, 258 (1960).
[2] Dutton, R. W., Dutton, A. H., and Vaughan, J. H., *Biochem. J.*, **75**, 230 (1960).
[3] Urso, P., and Makinodan, T., *Fed. Proc.*, **20**, 25 (1961).
[4] Baney, R. N., Vazquez, J. J., and Dixon, F. J., *Fed. Proc.*, **20**, 28 (1961).

PHARMACOLOGY

Effect of Derivatives of L-Cysteine on Growth of *Leuconostoc mesenteroides* (8042)

In the course of work on the chelating properties of certain amino-acid derivatives reaction products of cysteine with eight different aldehydes were prepared. The compounds were probably derivatives of thiazolidine-4-carboxylic acid[1–3]. They were prepared by previously described methods of synthesis or modifications of such methods[4–6]. In Table 1 the compounds are named simply by adding the name of the aldehyde to cysteine.

Table 1. Effect of Derivatives of L-Cysteine on Growth of *Leuconostoc mesenteroides* (8042)

μgm. cysteine, equivalent per tube	0·1 *N* sodium hydroxide titre (counts) in a potentiometric titrator					
	0	2	4	6	8	10
Cysteine hydrochloride	13	30	50	67	85	95
Cystine	14	34	55	78	99	112
Cysteine-acetaldehyde	13	27	44	65	80	93
,, propylaldehyde	12	32	51	73	88	94
,, butyraldehyde	13	29	49	69	85	102
,, isobutyraldehyde	16	26	44	66	82	105
,, benzaldehyde	13	26	43	54	73	88
,, formaldehyde	11	19	33	47	64	76
,, arabinose	12	19	33	43	59	65
,, glucose	12	21	33	42	53	71

By the courtesy of O. R. Brækkan, head of the Government Vitamin Laboratory, Bergen, Norway, I was given the opportunity to have the compounds tested as cysteine sources for *Leuconostoc mesenteroides* (8042) by the method described by G. D. Shockman *et al.*[7]. This organism is widely used for the microbiological determination of both cysteine and methionine. The results of one test are given in Table 1.

It is seen that the compounds fall into two distinct groups. The reaction products of cysteine with acet-, propyl-, butyr- and isobutyr-aldehydes were equally well utilized as were cysteine hydrochloride and cystine, while the products formed with formaldehyde, arabinose and glucose were less well utilized.

Cysteine-benzaldehyde was intermediate between these groups. The results were confirmed for cystine, cysteine hydrochloride, cysteine-formaldehyde, cysteine-butyraldehyde and cysteine-glucose using the same method, and for all the substances listed in Table 1 by a turbidimetric technique. In the latter test the concentrations used were doubled as compared with those used in Table 1.

These results probably have some bearing on the problem of determining cysteine in the presence of glucose[8–10], and may partly explain why Shockman *et al.*[7] get better results in microbiological cysteine (cystine) determinations when they autoclave the glucose separately before adding it to the sterile medium. Cysteine-glucose is readily formed in aqueous solutions[6,11]. The present results indicate that other aldehydes may probably also influence the determination of cysteine.

A. B. K. Njaa

Department of Pharmacology,
University of Bergen,
Norway.

[1] Schubert, M. P., *J. Biol. Chem.*, **111**, 671 (1935).
[2] Ratner, S., and Clarke, H. T., *J. Amer. Chem. Soc.*, **59**, 200 (1937).
[3] Schubert, M. P., *J. Biol. Chem.*, **130**, 601 (1939).
[4] Schubert, M. P., *J. Biol. Chem.*, **114**, 341 (1936).
[5] Debey, H. J., Mackenzie, J. B., and Mackenzie, C. G., *J. Nutr.*, **66**, 607 (1958).
[6] Weitzel, G., Engelmann, J., and Fretzdorff, A. M., *Z. physiol. Chem.*, **315**, 236 (1959).
[7] Shockman, G. D., Kolb, J. J., and Toennies, G., *Anal. Chem.*, **26**, 1657 (1954).
[8] Weller, L. E., Luecke, R. W., and Sell, H. M., *Proc. Soc. Exp. Biol. Med.*, **77**, 3 (1951).
[9] Riesen, W. H., Spengler, H. H., Robblee, A. R., Hankes, L. V., and Elvehjem, C. A., *J. Biol. Chem.*, **171**, 731 (1947).
[10] Camien, M. N., and Dunn, M. S., *J. Biol. Chem.*, **183**, 561 (1950).
[11] Agren, G., *Acta Physiol. Scand.*, **1**, 105 (1940).

Second-Generation Toxicity of Malathion in Rats

In a search for evidence of chronic toxicity of malathion, a breeding experiment was performed with rats under continuous exposure of all old and young animals to this insecticide. Malathion is an organophosphorus compound and is regarded as a relatively safe member of the anticholinesterases[1,2] so that it is widely used not only in agriculture but also in home gardening.

The experimental animals were Wistar rats bred for many years in this laboratory. The control rats received ground Purina Fox chow (Ralston Purina Company of Canada, Ltd., Woodstock, Ontario). The experimental animals were given the same food thoroughly mixed with malathion (technical grade, 95 per cent) purchased from the Niagara Brand Spray Co., Burlington, Ontario. In preparing the poisoned diet, 9 ml. of malathion were dissolved in 45 ml. of corn oil and gradually added in a mechanical mixer to 2·65 kgm. of the chow; there was thus 4,000 mgm. of malathion/kgm. of chow and the daily intake approximated 240 mgm. of malathion/kgm. of rat.

The experiment began with rats aged between 70 and 100 days. The colony consisted of 160 rats, equally divided between controls and experimental animals; there were thus 40 males and 40 females receiving malathion. These rats have now been fed for 5 months. They showed a normal rate of growth and at no time showed any sign of intoxication.

Ten weeks after the start of the experiment, eighteen females of the controls and eighteen females of the treated group were selected at random for breeding and were isolated from the main colony; twelve males were also taken from each group. The mating cages contained three females and one male; the male stayed in the cage for one week and was then replaced by another male that stayed for the same period of time. The outcome of this experiment is shown in tabular form (Table 1). Two of the treated rats had only four young each, a rather unusual number for the breed of this laboratory. The average litter sizes and their standard errors tended to be different between the groups, but they were statistically not distinguishable.

Table 1. Results of a Breeding Experiment in Rats treated continuously with Malathion mixed into the Food

	Control	Treated	χ^2	P
No. of females mated	18	18		
No. of females conceived	13	14		
No. of new-born	116	107		
Average litter size ± *S.E.*	10·5 ± 0·47	8·9 ± 0·68		~ 0·07
No. of new-born alive after 7 days	105	56	38·54	< 0·001
No. of new-born alive at weaning (21 days)	75	34	22·78	< 0·001

With the purpose of conducting breeding experiments of the second generation, all treated animals that weighed less than 30 gm. at the time of weaning were discarded. This left 26 of 34 surviving animals which were then matched by 26 control animals of approximately equal weight. The average weight of both groups was thus close to 44 gm. Nine weeks after birth, the average body-weights of the controls was 152 gm., those of the treated rats 136 gm. This evidence for the retardation of growth in the treated group is significant at the 1 per cent level.

The general state of health of the rat colony was good, since no deaths have occurred among the adult rats since the start of the experiment. However, a number of the young rats in the breeding experiment had ring-tail disease. A count at weaning time showed that 18 of 75 (24 per cent) of the control rats but 16 of 34 (47 per cent) of the treated rats had this disease. These percentages are different, the statistical significance being at the 5 per cent level.

This could mean that the drug has merely sensitized the animals to succumb to infection or other noxious influences. In any event, the results are not necessarily a direct consequence of cholinesterase inhibition: (1) the pseudocholinesterase activity of the plasma of the chronically treated rats was tested and found to be slightly elevated; this agrees with the finding that an accumulation of cholinesters induces cholinesterase formation in tissue culture[3]. (2) Malathion is never chemically pure and the purest preparations tend to be the least dangerous in acute toxicity work[4].

Two recent comprehensive reviews[1,2] make no reference to breeding experiments under exposure to organophosphorus compounds. The only comparable work reported in the literature seems to be that by Barnes and Denz[5], who found a high rate of death in the third generation of rats constantly exposed to *E* 605 (parathion). The recent observation of a mutagenic effect of organophosphorus compounds in *Drosophila melanogaster* deserves notice[6]. Depression of rate of growth by malathion has been seen in chicks[7]. There is also some evidence for the inhibition of growth by antagonists of acetylcholine: atropine, procaine, and tetraethylammonium prevent the regeneration of the forelimb in newts[8], and an effect of nicotine might be responsible for the decreased size of the babies of mothers who smoke[9].

Even with the present widespread use of insecticides, most human beings are exposed to not more than traces of these agents, while large doses were used in this work on rats, and even more so in the investigation on *Drosophila* quoted[6]. Nevertheless, it would appear that broad investigations of growing cells and insecticides are to be encouraged.

This work was supported in part by a grant 605–7–146 from the Ontario Department of Health.

W. Kalow
A. Marton

Department of Pharmacology,
University of Toronto.

[1] Holmstedt, B., *Pharmacological Rev.*, **11**, 567 (1959).
[2] O'Brien, R. D., *Toxic Phosphorus Esters*, 320 (Academic Press, New York, 1960).
[3] Burkhalter, A., Featherstone, R. M., Schueler, F. W., and Jones, M., *J. Pharm. Exp. Therap.*, **120**, 285 (1957).
[4] Hazleton, L. W., and Holland E. G., *A.M.A. Arch. Indust. Hyg.*, **8**, 399 (1953).
[5] Barnes, J. M., and Denz, F. A., *J. Hyg.*, **49**, 430 (1951).
[6] Rapoport, I. A., and Kostyanovskii, R. G., *Doklady Akad. Nauk S.S.S.R.* (translated issue, *Amer. Inst. Biol. Sci.*, **131**, 246 (1960)).
[7] Ross, E., and Sherman, M., *Poult. Sci.*, **39**, 1203 (1960).
[8] Singer, M., Davis, M. H., and Arkowitz, S., *J. Embryol. and Exp. Morph.*, **8**, Pt. 2, 98 (1960).
[9] Lowe, C. R., *Brit. Med. J.*, ii, 673 (1959).

5-Hydroxy-3-indoleacetamidine: a New Type of Blocking Agent against 5-Hydroxytryptamine

The possible role of 5-hydroxytryptamine (5-HT) regarding its action on the mammalian nervous system has been discussed repeatedly. However, pharmacological investigations were not very successful in clarifying this role. The well-known approach of using 'specific' antagonists against the neural effects of 5-HT has been stranded on two grounds: (*a*) the commonly known blocking agents of 5-HT are in fact only antagonists of 5-HT on smooth muscles, and (*b*) the actions of 5-HT on nervous receptors, except for a few peripheral sensory and ganglionic receptors, are not adequately explored. Recent investigations[1] demonstrate that even those agents which were shown before to block certain hypothetical nervous receptors of the guinea pig ileum[2] are of fairly low potency and/or selectivity to the ganglionic stimulant action of 5-HT. The tachyphylaxis observed in the ganglionic action of 5-HT suggested the use of compounds structurally closely related to 5-HT as its possible antagonists. One of these compounds, 5-hydroxy-3-indoleacetamidine (5-HTA), which was first described as a 5-HT-like stimulant agent[3], showed the following unique characteristics:

(1) In accordance with the data of Woolley and Shaw[3] 5-HTA was found to have pressor action on the nembutalized dog. It also stimulated the respiration markedly after intravenous injection of 10–20 μgm./kgm. Eighty μgm./kgm. intravenously blocked the pressor, reflex-bradycardic and respiratory stimulant actions of 5-HT for a few minutes. 5-HT itself, up to 160 μgm./kgm., was unable to influence significantly the actions elicited by 20–40 μgm./kgm. 5-HTA.

(2) A stimulant action of 5-HTA was found on the pelvic nerve-bladder preparation of the dog, but this action only partly resembled that of 5-HT. Given in doses of 1–4 μgm./kgm. intra-arterially 5-HTA evoked a twitch-like rapid contraction. 5-HT in the same dose-range produced a twitch-like reaction at first, followed by a slow contractile second phase[4]. The action of 5-HTA and the first phase of the 5-HT action were not inhibited by 0·2 mgm./kgm. bromo-lysergic acid diethylamine. Morphine, which is known to block nervous receptors sensitive to 5-HT (refs. 2, 4, 5), was found to be effective against 5-HT and 5-HTA in doses of 1–2 mgm./kgm. No blockage against 5-HT has been observed on the bladder with the small doses of 5-HTA (1–4 μgm./kgm.) used.

(3) On the isolated rat uterus 5-HTA up to 0·5 μgm./ml. did not show stimulatory action, whereas 0·01 μgm./ml. 5-HT stimulated the uterus. 5-HTA in the concentration mentioned diminished the action of 5-HT only slightly (dose-ratio: 4).

(4) Intra-arterial injections of 5–20 μgm. 5-HT and 5-HTA produced contractions of the cat nictitating membrane ('Nembutal' anæsthesia). With temporary occlusion of the external carotid artery, the threshold dose of 5-HT was lowered 2–3 times. With this condition the stimulation of the membrane is mainly produced indirectly by stimulating the superior cervical ganglion[5]. In contrast, the threshold dose for 5-HTA was rather increased by occlusion, indicating that its stimulant action was chiefly a direct effect on the nictitating membrane. 4–20 μgm. 5-HTA considerably diminished the ganglionic stimulation produced by 5–10 μgm. 5-HT (occluded external carotid artery). The same doses of 5-HTA were less or not effective against the direct stimulatory action of 5-HT on the nictitating membrane (open external carotid artery).

(5) On the inferior mesenteric ganglion preparation of the cat[6] no change or slight increase of the spontaneous post-ganglionic action potentials has been observed by intra-arterial injections of 1–20 μgm. 5-HTA. The ganglionic stimulant action of 2·5–10 μgm. 5-HT, however, was reduced by 1 μgm. and inhibited by 5–20 μgm. 5-HTA given 30 sec. and 4 min. before 5-HT. The stimulating action of 1,1-dimethyl. 4-phenyl-piperazinium iodide was unaltered by the same doses of 5-HTA.

5-HTA is the first compound which shows considerable antagonism against the ganglionic actions of 5-HT. Whereas it has stimulant properties on certain organs (urinary bladder, nictitating membrane) it usually lacks stimulant effects on the superior cervical and inferior mesenteric ganglia in the dose-range used. So far as its relative potency is concerned, 5-HTA is at least twenty times as potent as either LSD or bromo-LSD, cocaine and morphine against the ganglionic stimulant action of 5-HT. It is also more potent than bromo-LSD in preventing the reflex respiratory stimulant action of 5-HT. The blocking action of 5-HTA to 5-HT on the rat uterus is weak, inferior to that of LSD, bromo-LSD, chlorpromazine and of many other 'smooth muscle' type 5-HT antagonists. The compound seems to have a high degree of selectivity to certain nervous receptors sensitive to 5-HT.

Laszlo Gyermek

Geigy Research Laboratories,
Ardsley,
New York.

[1] Bindler, E., and Gyermek, L., *Fed. Proc.*, **20**, 319 (1961).
[2] Gaddum, J. H., and Picarelli, Z. P., *Brit. J. Pharmacol.*, **12**, 323 (1955).
[3] Woolley, D. W., and Shaw, E., *J. Pharm. and Exp. Therap.*, **121**, 13 (1957).
[4] Gyermek, L., *The Pharmacologist*, **2**, 89 (1960).
[5] Trendelenburg, U., *Brit. J. Pharmacol.*, **12**, 79 (1957).
[6] Herr, F., and Gyermek, L., *J. Pharm. and Exp. Therap.*, **129**, 338 (1960).

HISTOCHEMISTRY

Variation in Lyoglycogen in Starved and Normal Guinea Pigs

It has been shown that the histochemical staining reaction for tissue glycogen depends on the amount of lyoglycogen present in the tissues[1]. The present communication is a preliminary report of the nature of lyoglycogen. Lyoglycogen was obtained from the liver of six guinea pigs, two of which had been starved for 72 hr. and others that had had a normal diet. Hydrolysis of the resultant samples of lyoglycogen was carried out by refluxing with $N/1$ sulphuric acid at 100° C. for 8 hr. The acid solution was neutralized with barium carbonate, filtered and concentrated *in vacuo*. The solutions were applied to Whatman 3 *MM* papers which were developed in *n*-propanol–ethylacetate–water for 15–18 hr. Locating-reagents used were aniline-diphenylamine and silver nitrate.

Glycogen will yield glucose and small quantities of isomaltose on hydrolysis; sometimes other oligosaccharides are present due to acid reversion. The presence of isomaltose in hydrolysates of glycogen is taken to indicate 1–6 linkages in that molecule.

Fig. 1 shows a typical chromatogram. Samples of glycogen from the animals that were well fed yielded glucose and isomaltose in the hydrolysate. Although no quantitative estimates of the amount of isomaltose present in the glycogen hydrolysates from the well-fed animals were made, some indication of the amount of isomaltose present compared with the amount of glucose present may be given by the depth of staining on the chromatogram. The yield of isomaltose from these samples of glycogen seems to be far in excess of the expected quantity or of any that could be produced by acid reversion, consequently the number of 1–6 linkages in the parent glycogen would be greatly increased and may be identifiable with that part of the glycogen molecule or separate polysaccharide that Rosenfeld[2] described as having 33–47 per cent of 1–6 linkages.

The glycogen from the starved animals yielded only glucose. The absence of isomaltose in the hydrolysate obtained from the starved animals suggests a basic difference in the structure of the glycogen molecule in these conditions in that 1–6 linkages are absent.

From this preliminary work on glycogen that can be detected histochemically, it seems that the nutritional state of the animal has some influence on the structure of the glycogen molecule.

We thank Dr. W. J. Wheelan for his valuable suggestions and his gift of the disaccharide isomaltose.

W. J. C. Wilkinson
J. H. Kugler

Department of Anatomy,
The University,
Sheffield, 10.

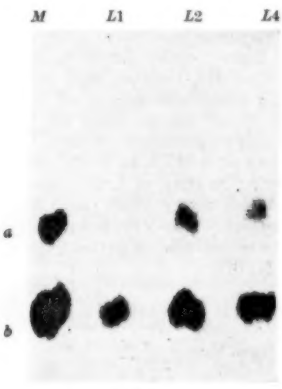


Fig. 1

Fig. 1. *M*, marker; *a*, isomaltose; *b*, glucose; *L1*, glycogen hydrolysate from starved animal (liver glycogen 242 μgm./100 mgm. liver wet weight). *L2*, *L4*, glycogen hydrolysate from fully fed animals (*L2*, liver glycogen 1,130 μgm.; *L4*, liver glycogen 1,012 μgm./100 mgm. liver wet weight). Note absence of isomaltose in *L1* and excessive amounts of isomaltose in *L2* and *L4*

[1] Kugler, J. H., and Wilkinson, W. J. C., *J. Histochem. Cytochem.*, **8**, 195 (1960).
[2] Rosenfeld, E. L., *Biokhimiya*, **23**, 877 (1958).

HÆMATOLOGY

Specific Antigens of Human Leucocytes and Thrombocytes

Attempts have been made to classify the specific antigens of human white blood cells and platelets, by using immune sera prepared in rabbits[1,2], sera from pregnant multiparous women[3,4] or from patients receiving multiple transfusions[5,6]. The different methods used by the various authors led often to inconsistent results.

In the present work immune sera from rabbits, monkeys and multiparous pregnant women have been used and compared using different serological methods.

Results obtained with rabbit anti-human leucocyte and thrombocyte sera showed the presence of common antigenic constituents of human erythrocytes, leucocytes and thrombocytes. These species specific antibodies of low titre (1/20–1/80) could easily be removed through appropriate absorptions. Common antigenic properties of thrombocytes and leucocytes of various species could also be observed. These antibodies, which were cell-specific for man, rabbit, mouse, sheep, guinea pig and rat, could be absorbed easily.

The titres obtained in rabbits against human leucocytes and thrombocytes were 1/640–1/1,280. The anti-leucocyte sera prepared against the leucocytes of a single donor reacted with leucocyte samples from every other human donor although with varying intensity, which may be due to differences in the agglutinability of the cells, with no correlation to their antigenic structure. The absorption of the serum with cells of any donor abolished completely the titre against all other donors. Similar results were obtained with thrombocytes.

The immune rabbit sera reacted strongly using the direct agglutination, agar-gel precipitation and tanned cell hæmagglutination tests. No correlation has been found between the intensity of agglutination of the leucocytes and the thrombocytes of the same donor.

The reactions obtained with the monkey anti-human leucocyte and anti-thrombocyte sera were similar to those obtained with the rabbit sera using the direct agglutination technique. The monkey antisera gave weaker reactions with the agar precipitation and the tanned cell hæmagglutination techniques.

The results obtained with sera of multiparous pregnant women differed significantly from those obtained with the animal anti-sera. Only direct

agglutination could be used. Agar-gel diffusion was negative and tanned-cell hæmagglutination weakly positive. Sera of five hundred women during the second half of their pregnancy were examined. All women had been pregnant between five and fifteen times. Fifty sera (10 per cent) were found to contain leuco-agglutinins in a titre of at least 1/10. Two sera were found to contain thrombo-agglutinins of a similar titre. These positive sera were examined each against leucocyte and thrombocyte samples obtained from thirty-six different blood donors[7]. Only compatible blood donors were used, most of them being of group *O*.

Table 1 summarizes the results obtained with fourteen different leucocyte samples when examined against ten different human anti-leucocyte sera. It can be seen that some of the sera as well as some of the cell samples show antigenic similarity. This similarity was especially well pronounced when whole families were investigated.

Table 1. AGGLUTINATION OF LEUCOCYTE SAMPLES BY TEN DIFFERENT HUMAN ANTI-LEUCOCYTE SERA

Donors of leucocytes	Human anti-leucocyte serum No. I	II	III	IV	V	VI	VII	VIII	IX	X
1	+	−	+	−	−	−	+	+	−	+
2	+	−	+	+	−	+	+	+	−	+
3	+	−	+	−	−	+	+	+	−	−
4	+	−	+	−	−	−	+	+	−	+
5	+	−	+	−	−	+	+	+	−	+
6	+	−	+	−	−	+	+	+	−	−
7	+	−	+	+	−	−	+	−	−	+
8	+	+	+	+	+	+	+	+	+	−
9	+	−	+	+	−	−	−	−	−	+
10	−	+	−	+	+	+	−	−	−	+
11	+	+	+	+	+	+	+	+	+	+
12	+	−	−	+	−	+	+	+	−	+
13	+	+	−	+	−	+	+	+	+	−
14	+	−	−	−	+	+	+	+	+	−

In Table 1 donors Nos. 1, 2, 3, 4 and 5 are brothers. No. 6 is the father and No. 7 is the mother. Nos. 13 and 14 are also brothers of another family.

This communication describes part of the work for a Ph.D. thesis carried out by Mrs. Gilboa-Garber at the Hebrew University, Jerusalem, and was supported in part by a grant from the Leukemia Research Foundation, Chicago.

NECHAMA GILBOA-GARBER
D. NELKEN

Department of Clinical Microbiology,
Hebrew University–Hadassah
Medical School,
Jerusalem, Israel.

[1] Butler, J. J., *J. Lab. Clin. Med.*, **55**, 110 (1960).
[2] Milgrom, N., Seligmann, M., Bernard, J., and Grabar, P., *Revue d'Hématologie*, **14**, 309 (1959).
[3] Payne, R., and Rolfs, M. R., *J. Clin. Invest.*, **37**, 1756 (1958).
[4] Van Rood, J. J., Eernisse, J. G., and van Leeuwen, A., *Nature*, **181**, 1735 (1956).
[5] Brittingham, T. E., *Vox Sanguinis*, **2**, 242 (1957).
[6] Dausset, J., *Acta Hæmat.*, **20**, 155 (1958).
[7] Nelken, D., Gilboa-Garber, N., and Gurevitch, J., *J. Clin. Path.*, **13**, 266 (1960).

PATHOLOGY

Cultivation of Monolayer Cultures of Frog Renal Cells

CONSIDERABLE evidence has been accumulated by Lucké[1], Duryee[2], Rose[3], Tweedell[4], and others for an agent which induces an organ-specific adenocarcinoma in the kidney of the leopard frog, *Rana pipiens*. Lucké[1] has postulated that this agent is probably of a viral nature. Although a percentage of the animals develop palpable renal tumours after a long and variable period of time following injection of tumour material, it is still desirable to devise other methods to propagate and possibly isolate the infective agent. At the same time, the large dimensions of the frog cells make them an ideal system with which to study the initiation of any subcellular modifications following incubation of cells with the tumour-inducing agent.

A procedure has been developed to provide an adequate *in vitro* host system. Adult frog kidneys, normally relatively sparse, slow-growing tissues in culture[5], are treated with trypsin by a method modified from that of Youngner[6] and Bodian[7], both using mammalian cells. The resulting cell suspension produces extensive cell sheets.

The animals, after being pithed, are perfused with calcium-free amphibian Ringer's solution (sodium chloride, 0·66 gm.; potassium chloride, 0·015 gm.; dextrose, 0·02 gm.; phenol red, 1·00 mgm.; penicillin, about 500 units per ml.; double glass-distilled water, 100 ml.; and sufficient 0·15 *M* sodium bicarbonate to make a final *p*H of 7·6) through the still-beating hearts of the animals to rid the kidneys of a majority of the trapped red blood cells by the resulting dilution of the blood. The organs are then dissected, minced, and washed twice in calcium-free amphibian Ringer's solution at room temperature. To ensure proper digestion of the kidneys, the organs are kept for 24 hr., or overnight, at 2°–4° C. in a fresh 0·25 per cent trypsin (Difco 1 : 250) solution in calcium-free Ringer's solution (*p*H 7·8) after 1 hr. exposure to the trypsin solution at room temperature before cooling.

The treated tissue, following the refrigeration period, is returned to room temperature and placed in a fresh trypsin solution (about 30 ml. per kidney) and is stirred magnetically for 2–3 hr. The cell suspension thus obtained is centrifuged for 5 min. at 1,000*g*, the supernatant trypsin medium discarded and replaced with calcium-free amphibian Ringer's solution, 1 ml. for each kidney. The suspension usually contains approximately 10^6 cells per ml. per kidney. Culture dishes are seeded with a final concentration of 500,000 cells per ml. of culture medium. The culture dishes then are sealed air-tight. The composition of the culture medium is: nutrient medium NCTC 109, diluted by adding 54 ml. of double-distilled water with 100 ml. of NCTC 109, 10 ml.; calf serum, 2·0 ml.; chick embryo extract, 2·0 ml.; 5 per cent lactalbumin hydrolysate in amphibian Ringer's solution, 1·0 ml.; glutamine (100 m*M*), 0·2 ml.; penicillin, about 500 units per ml. of medium.

Extensive cell sheets form within two to three days when the cultures are kept at 25° C. The cultures then are washed and fed with the above culture medium without lactalbumin hydrolysate. The monolayer sheets have been maintained, by feeding every three or four days, in good condition for several months. Attempts are now being made to derive a cell line from these frog kidney cultures.

Successful eye implants have been obtained from the cultured cell sheets which previously had been incubated with a crude extract of frog renal tumour, with resulting growth of the implant in the intraocular region. This preliminary evidence suggests that cellular transformations may have taken place, since normal kidney tissue implanted in the eyes of frogs seldom, if ever, exhibits any growth.

This investigation was supported in part by a Public Health Service post-doctoral research fellow-

ship, *CF*–12,861, from the National Institutes of Health, U.S. Public Health Service.

Walter Auclair

Department of Pathology,
George Washington University
School of Medicine,
Washington 7, D.C.

[1] Lucké, B., *J. Exp. Med.*, **68**, 457 (1938); *Ann. N.Y. Acad. Sci.*, **54**, 1093 (1952).
[2] Duryee, W. R., *Ann. N.Y. Acad. Sci.*, **63**, 1280 (1956).
[3] Rose, S. M., *Ann. N.Y. Acad. Sci.*, **54**, 1110 (1952).
[4] Tweedell, K. S., *Cancer Res.*, **15**, 410 (1955).
[5] Lucké, B., *J. Exp. Med.*, **70**, 269 (1939).
[6] Youngner, J. S., *Proc. Soc. Exp. Biol. Med.*, **85**, 202 (1954).
[7] Bodian, D., *Virology*, **4**, 575 (1956).

Acylase Activity of Ascites Tumour Cells on Dichloroacetyl Derivatives of Amino-acids

While investigating the specificity of acylase activity of soil bacteria on acyl derivatives of amino-acids[1–3], we noted that the acetone powder of Ehrlich ascites carcinoma cells was able to hydrolyse dichloroacetyl-DL-aspartic acid but not (or only to a small extent) the dichloroacetyl derivatives of some amino-acids such as DL-glutamic acid, DL-serine, DL-methionine and DL-phenylalanine[4].

We have now investigated the acylase activity of lyophilized powder of ascites tumour cells such as Ehrlich ascites carcinoma, sarcoma 180, leukæmia *SN* 36, ascites hepatoma *MH* 134 and *MH* 129P in *C3H* mice, and *AH* 7974, *AH* 130, and Yoshida sarcoma in rats on dichloroacetyl derivatives of the following twelve amino-acids: DL-aspartic and DL-glutamic acids, glycine, DL-alanine, DL-valine, DL-leucine, DL-phenylalanine, DL-tryptophan, DL-serine, DL-threonine, DL-methionine, and ε-*N*-benzoyl-DL-lysine.

Lyophilized powder of ascites tumour cells was prepared as follows: intraperitoneally transplanted ascites tumour cells were aspirated at the fifth day after transplantation, centrifuged at 1,000 r.p.m. for 10 min., washed three times with phosphate buffer solution, and then lyophilized. It was confirmed that the cells contained almost 85–95 per cent tumour cells.

Five mgm. of lyophilized powder of ascites tumour cells were added to 1 c.c. of neutralized 0·1 *M* substrate and 1 c.c. of *tris* buffer at *p*H 8·2, and the mixture was incubated at 37° C. for 2 or 18 hr. in the presence of toluene, and then titrated by Grassmann–Heyde's method.

The results obtained in the experiments carried out, under comparable conditions, on the acylase activity of lyophilized powder of eight ascites tumours are presented in Table 1. Ehrlich carcinoma, sarcoma 180 and leukæmia *SH* 36 were all able to hydrolyse dichloroacetyl-DL-aspartic acid but not (or only to a small extent) the dichloroacetyl derivatives of the other eleven amino-acids. It is of interest that *MH* 134, *MH* 129*P*, *AH* 7974, *AH* 130, and Yoshida sarcoma differed from Ehrlich carcinoma, sarcoma 180, and leukæmia *SN* 36 in failing to hydrolyse dichloroacetyl-DL-aspartic acid. Incidentally, *MH* 134, *MH* 129P, *AH* 7974 and *AH* 130 were hepatoma. The low acylase activities of *AH* 7974 and *AH* 130 seem worth noting when considered together with the fact that the rat liver could hydrolyse dichloroacetyl-DL-aspartic acid[5].

Thus, our experiment showed that there is a significant difference among ascites tumours in their capability of hydrolysing dichloroacetyl derivatives of amino-acids, and that acylase activity may be available as a tool for studying biochemical specificity of living material[1–3].

We thank Dr. S. Tatsuoka and Dr. T. Kaziwara, of Takeda Research Laboratories, for kindly supplying lyophilized powder of ascites tumour cells.

Yukio Kameda
Katsuhiko Matsui
Yukio Kimura
Etsuko Toyoura

Laboratory of Antibiotics,
Faculty of Pharmacy,
Kanazawa University,
Japan.

[1] Kameda, Y., *et al.*, *Nature*, **169**, 1016 (1952); **170**, 888 (1952); **181**, 1225 (1958); **182**, 453 (1958).
[2] Kameda, Y., *et al.*, *Yakugaku Zasshi*, **78**, 202, 748, 754, 759, 763, 765, 767, 769 (1958); **80**, 362 (1960).
[3] Kameda, Y., *et al.*, *Chem. Pharm. Bull.*, **6**, 321, 394, 395, 441 (1958); **7**, 702, 785, 787, 789 (1959).
[4] Kameda, Y., Toyoura, E., Kimura, Y., Matsui, K., Omori, T., and Takeuchi, K., *Yakugaku Zasshi*, **77**, 1351 (1957).
[5] Kameda, Y., *et al.*, *Chem. Pharm. Bull.* (in the press).

BIOLOGY

Chloride Regulation in *Triops*

Little physiological work on the branchiopods has been reported because of their relative scarcity and the unpredictability of supplies of animals. Among the freshwater species Krogh[1] reports on some preliminary experiments with *Branchipus* and *Triops* (*Apus*), Panikkar[2] gives some figures for the blood concentration of *Chirocephalus*, and Fritzche[3] gives a blood concentration for *Daphnia*.

An opportunity arose for me to use a few specimens of *Triops cancriformis* (Bosc) bred in laboratory conditions by Mr. A. R. Longhurst. The level of

Table 1. Acylase Activity of Ascites Tumour Cells toward *N*-Dichloroacetyl Amino-acids

Substrate	Percentage of hydrolysed substrate for 2 and 18 hr. at 37° C.															
	Ehrlich carcinoma		Sarcoma 180		Leukæmia *SN* 36		*MH* 134		*MH* 129*P*		*AH* 7974		*AH* 130		Yoshida sarcoma	
	2	18	2	18	2	18	2	18	2	18	2	18	2	18	2	18
Dichloroacetyl-DL-aspartic acid	27	56	28	55	22	52	0	7	0	3	1	3	tr	3	1	tr
Dichloroacetyl-DL-glutamic acid	0	1	0	0	0	1	1	0	0	0	0	tr	tr	tr	0	0
Dichloroacetylglycine	0	3	2	3	0	1	1	3	0	1	tr	3	0	1	1	tr
Dichloroacetyl-DL-alanine	1	4	1	4	0	tr	1	5	0	1	tr	tr	tr	tr	1	4
Dichloroacetyl-DL-valine	0	tr	0	3	0	0	0	2	1	3	1	tr	1	tr	1	1
Dichloroacetyl-DL-leucine	0	tr	0	3	1	tr	1	4	0	3	tr	tr	1	0	0	tr
Dichloroacetyl-DL-phenylalanine	0	tr	0	4	1	1	0	1	1	1	0	1	tr	6	tr	3
Dichloroacetyl-DL-tryptophan	0	1	0	1	0	0	1	0	0	2	0	1	1	5	tr	3
Dichloroacetyl-DL-serine	0	tr	1	tr	0	1	0	tr	0	1	tr	3	1	3	0	tr
Dichloroacetyl-DL-threonine	tr	tr	0	tr	0	1	0	0	0	0	0	1	tr	tr	0	1
Dichloroacetyl-DL-methionine	0	3	1	tr	0	3	0	4	0	0	tr	1	1	tr	1	tr
α-*N*-Dichloroacetyl-ε-*N*-benzoyl-DL-lysine	0	2	0	1	1	1	0	0	0	0	1	2	1	0	0	1

The digests consist of 5 mgm. of lyophilized sample, 1 c.c. of neutralized 0·1 *M* substrate, and 1 c.c. of *tris* buffer at *p*H 8·2 at 37° C. Hydrolytic activity was measured by Grassmann–Heyde's alcoholic titration method.

blood concentration was measured and some simple experiments were made to determine the degree of osmoregulation which the animals could achieve. Whole blood was withdrawn from the large dorsal sinus with soda-glass cannulæ and the chloride concentration of measured volumes determined by a Conway microdiffusion method[4] using Copenhagen sea-water as a standard.

The estimate for blood chloride concentration for six fed animals kept in filtered lake water (4·8 m.moles chloride/kgm. water) was 42·7 ± 5·4 m.moles chloride/kgm. water (range, 34·8–48·4). If chloride represents about 80 per cent of the anion concentration, the figure for total concentration would be about 55 m.equiv./kgm. water. It is clear from this estimate that *Triops* can maintain a blood concentration appreciably higher than the medium, and can be compared with some of the other entomostracan crustaceans in fresh water (Table 1).

Table 1

Animal	Blood conc. m.equiv./l.	Method of estimation
Branchipus (ref. 1)	30	Vapour pressure
Chirocephalus (ref. 2)	77	Vapour pressure
Daphnia (ref. 3)	68	Freezing-point
Triops	55	Chloride

In *Branchipus* and *Chirocephalus*, the ability to maintain this concentration against the osmotic gradient was dependent on salt uptake from the food, since starved animals quickly lost control. To test the effects of starvation on osmoregulation, some specimens of *Triops* were put into a large volume of distilled water (40 l.). The animals were sampled at intervals and the distilled water changed twice daily for 6 days. The blood chloride concentration remained high even after 6 days in distilled water, except for two moulting animals (Fig. 1). Within 4 days two other animals had moulted and died. After 6 days, one of the two survivors was moulting and the other in a poor condition, although its blood concentration was still high.

A further experiment was made in which some specimens were put into a sea-water dilution hyperosmotic (68 m.eq./kgm. water) to the blood. It is clear that these animals have shown a very different reaction to this reversed osmotic gradient (Fig. 1). After only 3 hr., the blood concentration rose to equivalence with the medium, and remained at this level for 3 days without any indication of a subsequent control. At the end of this time the animals began to lose tone.

It can be concluded that in its normal environment, *Triops* maintains its blood more concentrated than the medium by excluding the osmotic inflow of water, that is, it is relatively impermeable, and that its salt requirements are met by food intake. In a slightly hyperosmotic medium, however, it is unable to control either the influx of salt or the osmotic efflux of water.

Moulting, and the associated breakdown of cuticle impermeability, seriously impair the mechanism of osmotic control so that animals in this condition quickly succumbed to an osmotic stress.

It would be of interest to know more about the physiology of osmoregulation in this species especially in relation to the function of the renal organ. This appears to be a large and complex structure, and perhaps one might predict that the animal has an efficient mechanism for salt resorption.

GWYNETH PARRY

Ministry of Agriculture, Fisheries and Food,
10 Whitehall Place, London, S.W.1.

[1] Krogh, A., *Osmotic Regulation in Aquatic Animals* (Cambridge Univ. Press, 1939).
[2] Panikkar, N. K., *J. Exp. Biol.*, **18**, 110 (1941).
[3] Fritzche, H., *Int. Rev. Hydrobiol.*, **8**, 22 (1917).
[4] Conway, E. J., *Microdiffusion and Volumetric Error* (London: Crosby Lockwood, 1951).

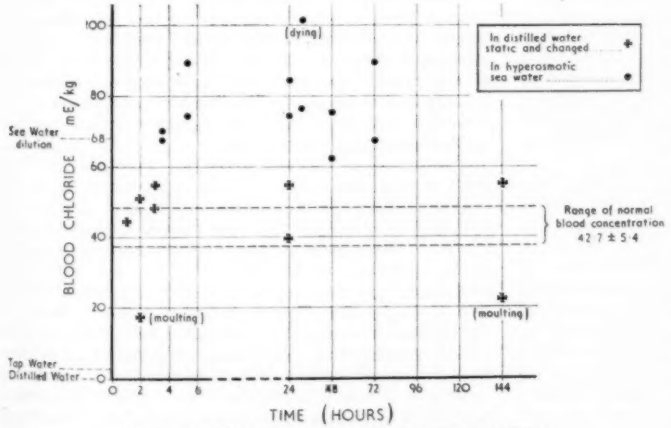


Fig. 1. Regulation of chloride in *Triops cancriformis* (Bosc)

Effect of Feeding Griseofulvin on Rate of Growth of Chicks

GRISEOFULVIN, originally isolated from *Penicillium griseofulvum* by Oxford[1] *et al.* in 1939, is produced commercially by the fermentation of three *Penicillium* species: *griseofulvum* Dierck X, *janczewskii* and *patalum*. Brian[2] *et al.* first described its antifungal properties in 1946, but another twelve years elapsed before Gentles[3] demonstrated its efficacy against *Microsporum canis* and *Trichophyton mentagrophytes* when administered orally to infected guinea pigs.

This discovery greatly stimulated interest in the clinical use of griseofulvin and many trials against a variety of dermatophytes have since been carried out[4].

Work on toxicity indicates that large doses injected intravenously in rats cause a transient depression of mitosis while intraperitoneal administration severely damages the seminal epithelium[5], but when taken orally, the toxicity of griseofulvin is low[6,7]. In view of the low oral toxicity and in the absence of any published reports on this aspect, it was decided to investigate the influence of griseofulvin in the diet on the rate of growth of chicks.

Four groups, each of 26 day-old broiler type cockerels, were used; two groups were fed commercial broiler crumbs, containing 5 mgm. procaine penicillin/lb., and the other two received the same basal diet supplemented with 5 mgm. griseofulvin/lb. On arrival the newly hatched chicks were distrib-

uted among the groups according to weight so that the average initial weights were constant, 40 gm. in this case. Rearing was carried out in wire-floored tier brooders and the chicks were weighed weekly over a period of four weeks. For the analysis of the results, regression coefficients of logarithm weight on time, based on the two weekly group means for each diet, were calculated and the significance of the difference between the regressions tested by means of a t-test in the usual way.

Table 1. Average Weights of Chicks (gm.)

Diets	Beginning of week: 1	2	3	4	5	Regression coefficients, diets on weeks
Control + griseofulvin	40·0	86·2	175·7	305·3	467·7	0·2693
Control	40·0	85·3	159·8	291·4	447·9	0·2636
Difference	—	0·9	5·9	13·9	19·8	0·0057
						$t = 2·33$, $P < 0·05$

From the results shown in Table 1 it is apparent that griseofulvin stimulated growth significantly throughout the period of the trial. The amount of food consumed per lb. of live weight was 1·98 lb. for the griseofulvin supplemented group and 1·95 lb. for the control group. The difference between these figures is not significant.

The assistance of I.C.I., Ltd., in providing facilities for this experiment is gratefully acknowledged.

W. P. Jaffe

School of Veterinary Science,
University of Bristol.

[1] Oxford, A. E., Raistrick, H., and Simonart, P., *Biochem. J.*, **33**, 240 (1939).
[2] Brian, P. W., Curtis, P. J., and Hemming, H. G., *Trans. Brit. Mycol. Soc.*, **29**, 173 (1946).
[3] Gentles, J. C., *Nature*, **182**, 476 (1958).
[4] Sulzberger, M. B., and Baer, R. L., *Excerpta Med.*, **13**, 145 (1959).
[5] Paget, C. E., and Walpole, A. L., *Nature*, **182**, 1320 (1958).
[6] Johnson, S. A. M., and Cameron, G. H., *Antibiotics Annual* 1959–60, 687 (1960).
[7] Greco, G. A., Moss, E. L., and Foley, E. J., *Antibiotics Annual* 1959–1960, 663 (1960).

Feeding of Bamboo Flowers to Laboratory Rats and Mice

According to a popular myth there is a baffling connexion between the flowering of bamboos and the appearance of rat hordes which spread around destroying crops and eating chickens, puppies and even babies[1]. The present inquiry is an attempt to explain the mysterious phenomenon of an abnormal increase in field rats associated with the flowering of bamboos as recently witnessed in North-east Frontier and Tripura areas. The problem was approached from the point of view of evaluating the palatability, the œstrogenic activity and the reproduction stimulating values of bamboo flowers for rats.

Small quantities of 'mooli' bamboo flowers and fruits and 'pularwa' bamboo flowers from the affected area were received in a semi-dried condition. On dry-matter basis the percentages of crude protein in these samples were 11·39, 14·34 and 13·90 and of crude fibre 39·10, 19·42 and 31·71 respectively compared to the values of 12·81 crude protein and 3·58 crude fibre in the stock diet of rats. The laboratory rats did not relish these substances *per se*; but their inclusion in the normal diet of groups of rats at 10 and 20 per cent levels showed that the total dry matter ingestion may be slightly stimulated at the lower level of incorporation.

An evaluation of the œstrogenic activity in 'mooli' flowers was carried out according to Bickoff *et al.*[2]. Groups of six immature mice each were fed the normal and experimental diets for a period of one week before they were killed for determining the weights of the freshly excised uteri. The results in Table 1 show that the œstrogenic activity in the 'mooli' flowers was practically nil at the levels tested.

Table 1. Uterine Weight Response of Immature Mice on Diets containing the Extract from Bamboo Flowers

Group No.	Addition to 100 gm. normal diet	Live weight (gm.)	Uterine weight (mgm.)	Difference compared to control
I	Nil	5·33 ± 0·61	3·01 ± 0·42	—
II	Stilbœstrol, 0·75 μgm.	5·50 ± 0·62	5·58 ± 0·63	Highly significant
III	Extract from 8 gm. of air-dried bamboo flowers	5·33 ± 0·61	3·20 ± 0·55	—
IV	Extract from 1·6 gm. of air-dried bamboo flowers	5·67 ± 0·76	3·30 ± 0·42	—

Direct evidence of the incorporation of bamboo flowers in the stock diet of rats on their reproductive performance was obtained with local bamboo (*Bambusa arundinaceae*) flowers. Six male and twelve female adult white rats were distributed in three identical groups. One group received the normal diet while the other two groups received diets in which shade-dried bamboo flowers were incorporated at 2 and 15 per cent levels, respectively. The male and female rats of each group were kept separately during the first 15 days of experimental feeding. They were then mated within the groups and continued on the scheduled diets until all the female rats brought forth litters. The average data are recorded in Table 2.

Table 2. Observations on the Reproductive Performance of Rats on Bamboo Flower Diets

Group No.	Level of bamboo flowers in normal diet (per cent)	Period from mating to littering (days)	Litter size	Weight per new-born rat (gm.)
I	Nil	30·00 ± 2·61	8·25 ± 1·44	4·88 ± 0·05
II	2	25·75 ± 1·25	8·00 ± 0·00	5·10 ± 0·11
III	15	36·75 ± 10·02	7·25 ± 0·85	4·80 ± 0·13

The differences between the control and experimental groups were statistically not significant in regard to the length of the period from date of mating to date of littering, litter size or the weight of the new-born rat. Evidently the incorporation of bamboo flowers in the stock diet under the conditions of the test did not have any stimulatory effect on the reproductive activity in rats.

The bamboo flowers were not palatable to rats even in the fresh state. The sugar content in the nectar of bamboo flowers is lower than in those of common honey plants like apple, dandelion and clovers[3]. Bamboo sprouts promote growth of pathogenic bacteria which barely grow on the basal medium[4], and the bamboo seed is superior to both rice and wheat in overall nutritive value[5]. Breeding disturbances in sheep in Australia have been related to the presence of œstrogenic substances in subterranean clover[6], but their high concentration in spring grasses in Great Britain has been without any reproductive disturbance in any species so far[7]. The search for œstrogenic substances in bamboo flowers was

prompted by their well-established occurrence in plant materials and their value as growth promoters[8]. The possibility that, as growth promoters, œstrogenic substances from diverse sources may have unrecognized useful potentialities may not be discounted.

The limited evidence presented here does not support the myth of increase in rat population as a direct result of the ingestion of bamboo flowers.

We are grateful to the Director, Indian Veterinary Research Institute, Izatnagar, for his interest in this work.

V. Mahadevan
S. S. Negi
O. N. Agarwala

Division of Animal Nutrition,
Indian Veterinary Research Institute,
Izatnagar, U.P.

[1] *The Observer, Hindustan Times Weekly* (April 10, 1960).
[2] Bickoff, E. M., Booth, A. N., Livingston, A. L., Henderikson, A. P., and Lyman, R. L., *J. Animal Sci.*, **18**, 1000 (1959).
[3] Shaw, F. R., *Gleanings Bee Cult.*, **81**, 88 (1953).
[4] Yoshida, N., and Ikejiri, S., *Shikoku Acta Med.*, **1**, 25 (1950).
[5] Rao, M. V. L., Subramanian, N., and Srinivasan, M., *Curr. Sci.*, **24**, 157 (1955).
[6] Biggers, J. D., and Curnow, D. H., *Biochem. J.*, **58**, 278, 283 (1954).
[7] Bartlatt, S., Folly, S. J., Rowlands, S. J., Curnow, D. H., and Simpson, S. A., *J. Animal Sci.*, **13**, 99 (1949).
[8] Bradbury, R. B., and White, D. E., *Vitamins and Hormones*, **12**, 207 (1954).

Classification of Fossil Microplankton

It is becoming increasingly desirable that a definite decision be made as to whether the fossil organic-shelled microplankton (dinoflagellates, hystrichospheres, and genera of presumed microplankton *incertae sedis*) should be classed, for nomenclatural purposes, in the animal or in the plant kingdom. The dinoflagellates, a group of unicellular organisms some of which contain chlorophyll, are clearly algae, while others contain no chlorophyll and are vagile and predatory, and are at present alternatively regarded as the class Dinoflagellata or class Dinophyceae. The hystrichospheres, typically marine in occurrence and defined by their morphology, are regarded as an order *incertae sedis*, the order Hystrichosphaeridia, and have been treated by most authors as animals: the majority are now known to be dinoflagellate cysts, while others, less capable of definite attribution, may be zygospores of desmids, eggs of copepods or other planktonic organisms, or even plant spores.

Much confusion about nomenclature must inevitably result when these organisms are indifferently classed in two kingdoms and may thus be dealt with under two different taxonomic codes. The proposed third kingdom, the Protista, to comprise all unicellular organisms, has not secured general recognition: it is highly unlikely that a third system of nomenclature will be set up. A choice must thus be made as to which of the existing systems should be followed, and whichever is decided on must be applied consistently if further confusion is to be avoided.

We consider that these groups should be classified as plants. The dinoflagellates surviving as fossils are relatively thick-shelled forms, often having an armour of distinct plates. Investigations of living plankton show that such forms are relatively sessile and contain chlorophyll: the vagile, predatory forms have very thin shells and are probably not capable of fossilization. Evitt[1] has demonstrated that a large majority of the hystrichospheres and allied forms *incertae sedis* possess an opening in the shell (a pylome) through which escape can take place after encystment and are quite certainly the abandoned cysts of dinoflagellates, a conclusion suggested also in many genera by similarities in overall morphology. For these forms, use of the Botanical Rules[2] would be greatly advantageous in that there is provision for the creation of form genera and species: the confusion that might result from application of the rule of priority, should the identity of a motile dinoflagellate with an encysted form be proved, may thus be avoided. No such provision exists in the Zoological Rules, the comparable vehicle of para-taxonomy[3] having been rejected by the International Committee in London, 1958.

Classification under the Botanical Rules conforms to a large degree with present practice. Deflandre, in his work on the fossil dinoflagellates, has classed them as Dinophyceae: the Russian authorities, such as Naumova, regarded all hystrichospheres as plants for taxonomic purposes. The majority of workers in this field are palynologists for whom the procedure here proposed would be considerably more acceptable.

We therefore propose that the fossil microplankton be regarded as subject to the Rules of Botanical Nomenclature, as applied to fossil plants: that, within the framework of this classification, fossil dinoflagellates in the motile stage be regarded as genera and species: fossil cysts of dinoflagellates as form genera and species: and hystrichospheres and other genera *incertae sedis*, for which a relation to the dinoflagellates has not been proved, as genera and species or form genera and species *incertae sedis*.

Until a full re-definition of the order Hystrichosphaeridia, on the basis of a more complete knowledge of their affinities, has been undertaken, we do not feel it desirable to alter the present grouping into families of the hystrichospheres. For the present, we consider that it would be adequate if these organisms were classed as plants and descriptions of them are formulated on this basis.

C. Downie
G. L. Williams

Department of Geology,
University of Sheffield.

W. A. S. Sarjeant

Department of Geology,
University College of North
Staffordshire,
Keele.

[1] Evitt, W. R., *On the Morphology of Fossil Dinoflagellates* (in the press, 1961).
[2] Lanjouw, J., *et al.*, International code of botanical nomenclature adopted by the Eighth Intern. Botanical Congr., Paris, July 1954 (Utrecht, 1956).
[3] Moore, R. C., and Sylvester-Bradley, P. C., *Bull. Zool. Nomencl.*, **16**, part 1/4, 1 (1957).

Alteration of Permeability of *Lactobacillus plantarum* caused by Chlorpromazine

The mechanism for penetration of oxalacetate into *Lactobacillus plantarum* 17–5 is inducible. Unless *L. plantarum* is grown for a few hours in a medium which contains either oxalacetate, acetoacetate or α-ketoglutarate, there is poor penetration of oxalacetate into cell suspensions of this organism afterwards prepared. Oxalacetate decarboxylase (which is also inducible) can be readily detected manometric-

ally in whole organism suspensions of *L. plantarum* grown with any of the three compounds already mentioned, but can only be detected in disrupted (acetone-dried) organisms if the bacteria had been grown with L-malate, a compound which induces synthesis of oxalacetate decarboxylase but does not induce the mechanism for oxalacetate to penetrate the cell[1]. An agent which increases cellular permeability, at least with respect to oxalacetate, may be detected by increase of oxalacetate decarboxylase activity of undisrupted *L. plantarum* which had been grown in a medium containing L-malate.

For the experiments reported here, *L. plantarum* was grown at 35° C. in a medium composed of yeast extract, glucose, and L-malate (1·0 gm./100 ml. each). Chlorpromazine was added to the growth medium after the organisms began to increase exponentially. The amount of chlorpromazine added varied within the range of 0·5–5·0 mgm./100 ml. final medium. Growth was allowed to continue for 3–6 hr. after addition of chlorpromazine.

Samples of each culture were examined for gross morphological changes and for differences in density of final growth. Cultures which had grown with less than 3·0 mgm. chlorpromazine appeared like the untreated control. There were fewer cells produced in cultures which contained more than 3·0 mgm. chlorpromazine.

Cultures grown either without or with 1·0 mgm. chlorpromazine were collected by centrifugation, washed and assayed for both oxalacetate decarboxylase and malic decarboxylase (malic enzyme) using methods previously described[1,2]. Since malic decarboxylase is also inducible, but can be readily detected in suspensions of undisrupted cells, changes in the activity of this enzyme served as a control that chlorpromazine inclusion in the growth medium did not quantitatively alter enzyme synthesis[2].

Only cell suspensions of bacteria which had been grown with chlorpromazine showed appreciable increase in oxalacetate decarboxylase activity (Table 1). There was no effect on malic decarboxylase activity of cell suspensions or of either oxalacetate decarboxylase or malic decarboxylase activity of acetone-dried cells.

Table 1. Effect of Chlorpromazine (CPZ) during Growth on Subsequent Enzyme Activity of *L. plantarum*

Source of enzyme	Treatment during growth	Decarboxylase measured	Per cent activity
Cell suspension	None	oxalacetate	10
" "	CPZ	"	100
" "	None	malate	100
" "	CPZ	"	100
Acetone-dried cells	None	oxalacetate	100
" "	CPZ	"	100
" "	None	malate	100
" "	CPZ	"	100

It has already been shown that one site of activity of chlorpromazine in *L. plantarum* involves diphosphophyridine nucleotide and is related to the ability of chlorpromazine to serve an electron donor[2,3]. Quite possibly, the alteration in membrane permeability caused by chlorpromazine in the experiments reported here also involves a site dependent on diphosphopyridine nucleotide. In other recent experiments we have shown that chlorpromazine increases membrane permeability of animal-type cells[4], and plant-type cells (Nathan and Zahalsky, in preparation). A preliminary survey of phenothiazines indicates that ability to alter membrane permeability is linked to ease with which free radicals are formed.

Chlorpromazine promises to be a useful tool for making materials in the surrounding milieu accessible to the inside of cells without injuring the cell to the extent that it cannot grow and reproduce.

This work was supported by a grant from the Peninsula Health Fund.

Helene A. Nathan

Department of Medicine,
Laboratory of Clinical Investigation,
Medical College of Virginia,
Richmond 19, Virginia,
and
Haskins Laboratories, Inc.,
New York.

[1] Nathan, H. A., *J. Gen. Microbiol.*, **17**, 415 (1961).
[2] Nathan, H. A., *Arch. Microbiol.*, **38**, 107 (1961).
[3] Nathan, H. A., *Proc. Fourth Intern. Cong. Clin. Chem.*, Edinburgh, 169 (1960).
[4] Nathan, H. A., and Friedman, W., *Proc. First Intern. Conf. Protozool., Prague* (1961).

A Chamber for culturing and collecting the Nematode *Panagrellus redivivus*

Panagrellus redivivus L., Goodey, 1945, is frequently the nematode chosen for laboratory work because of its size (maximum length of approximately 1·5 mm.) and the ease with which it can be cultivated[1]. This free-living worm has been employed for the evaluation of nematocides[2] and repellants[3], in metabolic studies[4], and to induce morphogenesis in predaceous fungi[5].

A constant supply of *P. redivivus* was necessary for work in this laboratory on the activities of fungi that capture and kill nematodes. To meet this requirement a chamber that served for both the culture and collection of *P. redivivus* was designed and employed. The apparatus (see Fig. 1) consisted of a funnel (15 cm. diameter, 60° angle) with a ground lip and attached standard-taper stopcock. A saddle (*A*) constructed of glass rod was placed in the funnel as support for a heavy Petri dish bottom (*B*) and approximately 25 gm. of oatmeal and 45 ml. of water

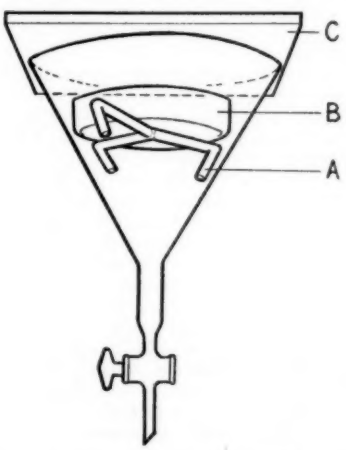


Fig. 1. Chamber for the culture and collecting of *P. redivivus*

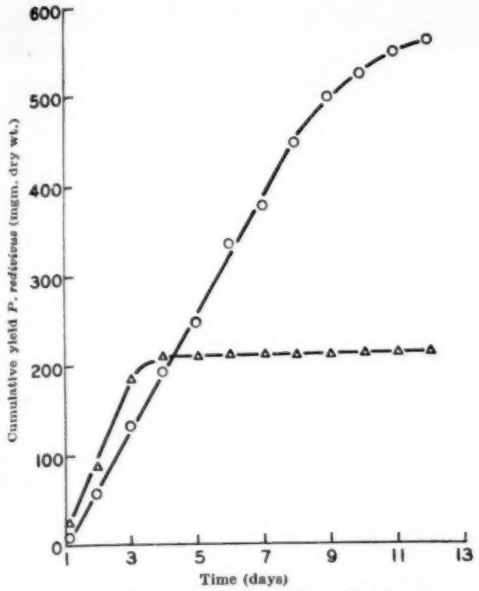


Fig. 2. Influence of temperature on the yield of *P. redivivus*. ○, 20° C.; △, 28° C.

were added to the dish (2 × 9 cm.). The stopcock was closed and water was then added to the funnel until the liquid-level was 3–4 mm. below the open end of the dish. The moist oatmeal was inoculated with *P. redivivus* and the unit was closed using a ground-glass plate (*C*) as lid. The dish was arranged in a manner such that it did not touch the wall of the funnel and was approximately 1·0 cm. below the lid.

P. redivivus multiplied rapidly and after 3–5 days at 28° C. or 7–8 days at 20° C. numerous adults and larvæ moved out of the oatmeal, over the edge of the dish and into the water contained in the funnel. Since the density of nematodes exceeds that of water, the worms were concentrated above the stopcock by sedimentation. The dry weight of nematode tissue yielded by chambers incubated at 20° and 28° C. was determined at daily intervals from the time of first harvest. The results, illustrated in Fig. 2, showed that 20° C. was the more suitable storage temperature. Although development of *P. redivivus* was more rapid at 28° C., worm production at that temperature ceased after 4 days. At 20° C. nematodes were produced for 12 days and the total yield exceeded 550 mgm. Since a dry weight of 1·0 mgm. was equivalent to approximately 30,000 individuals, more than 16×10^6 worms were collected in 12 days from each chamber incubated at 20° C. No attempt was made to collect or enumerate the many nematodes that remained in the oatmeal, but on prolonged incubation their numbers were depleted by sporadic migrations from the dish into the funnel.

The chamber as described is efficient and convenient and serves simultaneously for the culture and collection of *P. redivivus*. However, it can be sterilized or modified to meet specific requirements. The stem of the funnel can be refrigerated to maintain worms in a state of relative inactivity until collected. Moreover, the stem can be elongated and filled with glass beads. Worms collected after entering the funnel and working their way down the bead column to the stopcock are relatively free of contaminating micro-organisms.

We thank J. Postman and the New Brunswick Scientific Co. (New Brunswick, New Jersey) for construction of the chambers. This investigation was supported in part by National Science Foundation grants *G*5949 and *G*9749.

E. J. Winkler
David Pramer

Department of Agricultural Microbiology,
Rutgers, The State University,
New Brunswick, N.J.

[1] Tarjan, A. C., *Proc. Helminthol. Soc. Wash.*, **22**, 33 (1955).
[2] Taylor, A. L., Feldmesser, J., and Feder, W. A., *Plant Dis. Rep.*, **41**, 527 (1957).
[3] Davis, D., and Deak, J. E., *Plant Dis. Rep.*, **44**, 622 (1960).
[4] Santmyer, P. H., *Proc. Helminthol. Soc. Wash.*, **23**, 30 (1956).
[5] Tarjan, A. C., *Nature*, **185**, 779 (1960).

Periodic Acid–Schiff Technique for the Demonstration of Extra- and Intra-cellular Stages of Gregarine and Coccidian Protozoa

During a histological investigation of gut structure in the rhynchocœlan *Lineus ruber*[1] it was noted that both extra- and intra-cellular stages of the gregarine *Urospora nemertes*, which infected approximately 75 per cent of the specimens examined, stained intensely with the periodic acid–Schiff technique[2]. The intracellular stages, in particular, are difficult to demonstrate by routine staining methods, but after periodic acid–Schiff treatment they are strikingly prominent and may be readily located in sections with the low-power objective.

In view of the potential value to the protozoologist of this use of the periodic acid–Schiff technique its effectiveness with other gregarine species and the related group of parasitic protozoa, the Coccidia, was investigated. In all cases every stage in the life-history gave a strong positive reaction and the four species of coccidians examined (*Eimeria tenella*, *E. maxima*, *E. acervulina* and *E. necatrex*, from the gut of the domestic fowl) were readily located even when only small numbers were present in the infected tissue. Particularly striking results were obtained with the gametocyte stage (Fig. 1).

The choice of fixative does not appear to influence the reaction to periodic acid–Schiff to any significant extent and comparable results were obtained after

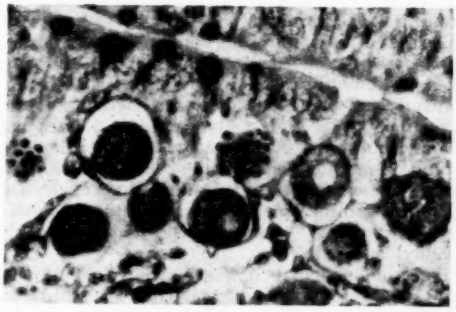


Fig. 1. Transverse section of fowl cæcum showing macro- and micro-gametocytes of *Eimeria maxima*. Trichrome periodic acid–Schiff (× 650)

fixation in Susa, 5 or 10 per cent neutral formalin or 90 per cent alcohol. If details of host tissue are required, the periodic acid–Schiff may be followed by the celestin blue/hæmalum and orange *G* method[3] to give a full trichrome stain.

I wish to thank Dr. Horton-Smith, Houghton Poultry Research Station, Houghton, Huntingdonshire, for supplying material infected with the four species of *Eimeria*.

J. B. Jennings

Department of Zoology,
The University,
Leeds 2.

[1] Jennings, J. B., *Biol. Bull.*, **119**, 189 (1960).
[2] Pearse, A. G. E., *Histochemistry, Theoretical and Applied*, second edit. (J. and A. Churchill Ltd., London, 1960).

ENTOMOLOGY

Evaluation of the Activity of Some Diphenyl Compounds on Winter Eggs of the Fruit Tree Red Spider

For several years some red spider ovicides have appeared on the market. Most of these compounds are active chiefly on the summer eggs, and far less active on the winter eggs of the fruit tree red spider *Metatetranychus ulmi* Koch. One of these compounds, 2,4,5,4′-tetrachlorodiphenyl sulphone (tetradifon), shows extremely high ovicidal and larvicidal activity on Tetranychids, but is not active on the winter eggs of *Metatetranychus ulmi*. In the course of investigation of the past five years a large number of diphenyl compounds has been tested on tetranychids[1]. Some of them have been examined for activity on the winter eggs and compared with some other compounds. The latter experiments are reported here.

Apple twigs bearing winter eggs of the fruit tree red spider were collected during winter and kept outdoors. Before treatment the twigs were cut into pieces of about 4 in. and then dipped into miscible oil–water emulsions. The treated pieces were put into water-filled vials. The whole was kept in climatically conditioned racks under continuous illumination at 20–22° C. and 80–90 per cent relative humidity.

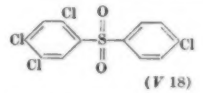
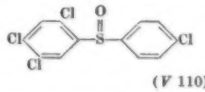
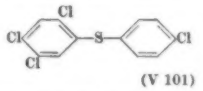
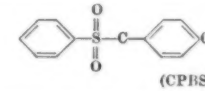
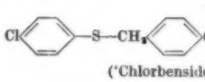
A serious difficulty of these laboratory experiments is the high percentage of untreated eggs that do not hatch. This percentage may vary between 30 and 80 after one month of incubation. Hatching is especially reduced if heavily infested twigs are used, probably as a result of overpopulation. Nevertheless, the differences between the various substances seem justified by their consistency under various levels of hatching in the batches.

The reduction of hatching shown in the tables refers to the percentages calculated after Abbott's formula. Table 1 shows the results of trials with tetrachlorodiphenyl compounds as compared with the known acaricides CPBS and 'Chlorbenside'. Tetrachlorodiphenyl sulphone is not active at all, whereas the sulphide homologue is highly active. The latter appears to be the best of the compounds examined.

Table 2 summarizes the results obtained with the trichloro-diphenyl compounds and tetrachlorodiphenyl sulphide.

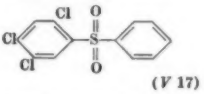
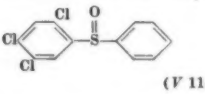
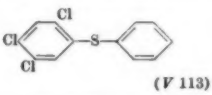
In a previous paper[2] it was shown that *V* 18, *V* 110 and *V* 101 were equally effective on eggs and larvæ of *Tetranychus urticae* Koch on bean plants. When dipping eggs of *Tetranychus urticae* Koch in emulsions of these compounds, however, *V* 18 shows the highest ovicidal activity. After hatching of the surviving eggs, the larvæ are killed by the deposit of all three compounds, so that the overall mortality is the same. But if leaves bearing eggs of *Tetranychus* at the under side are treated on the upper side only, the highest egg-kill is obtained by *V* 101, and the least kill by *V* 18, whereas *V* 110 takes an intermediate

Table 1. Reduction of Hatching of Winter Eggs of *Metatetranychus ulmi* Koch after dipping of Egg-bearing Apple Twigs in Emulsions. Mean Percentages of Three Experiments in 1957 and 1958

Compound	Concentration (p.p.m.)	Percentage reduction
 (*V* 18)	500 250 125	7 10 5
 (*V* 110)	500 250 125	7 14 0
 (V 101)	500 250 125	100 90 87
 (CPBS)	500 250 125	71 61 29
 ('Chlorbenside')	500 250 125	64 66 79

* One experiment.

Table 2. Reduction of Hatching of Winter Eggs of *Metatetranychus ulmi* Koch after dipping of Egg-bearing Apple Twigs in Emulsions. Mean Percentages of Three Experiments (1959)

Compound	Concentration (p.p.m.)	Percentage reduction
 (*V* 17)	1,000 300 100	0 0 0
 (*V* 114)	1,000 300 100	92 0 0
 (*V* 113)	1,000 300 100	98 61 0
V 101	300 100 30	100 98 97
50/50 mixture *V* 18/*V* 101	150/150 50/50 15/15	99 92 56

position. This result is evidently a consequence of the different penetration properties of the compounds.

In this respect it is interesting that the highest activity on winter eggs of *Metatetranychus* is shown by *V* 101, which also has the best leaf-penetration. In laboratory trials *V* 101 appears also more active than CPBS, which has been in use for the control of winter eggs of the fruit tree red spider for some years. The addition of *V* 18 does not seem to influence the action of *V* 101.

The same sequence of activity on the winter eggs is seen with the trichloro-compounds *V* 17, *V* 114 and *V* 113 (Table 2). Also in this case the sulphide homologue is more active than the sulphone, presumably as a result of the better penetration through the egg shell. The activity, however, is far less than with *V* 101.

J. Meltzer

Agrobiological Laboratory,
"Boekesteyn",
's-Graveland,
The Netherlands.

[1] Huisman, H. O., Uhlenbroek, J. H., and Meltzer, J., *Rec. Trav. Chim. Pays-Bas*, **77**, 103 (1958).
[2] Meltzer, J., and Dietvorst, F. C., *Proc. Fourth Int. Cong. Crop Prot., Hamburg*, 1957, 669 (1959).

A Technique for exposing Individual Flies on Insecticidal Deposits

A number of methods have been described for testing residual insecticides against various insects, and for the bio-assay of insecticide deposits on treated surfaces. In general these entail confining the insects for testing under a transparent container on the treated surface for a certain time of exposure, and measuring the mortality after holding in clean cages for a further period. Wiesmann[1] used halves of Petri dishes, while Hadaway and Barlow[2] used glass tubes, inverted funnels and 'Perspex' cylinders.

One of the difficulties common to these methods, particularly when testing flying insects, has been to ensure that the insect remains in contact with the treated surface for the whole of the time of exposure. According to the type of apparatus, one is advised to blow down the cylinder, or to rotate the tube in order to induce the insect to settle on the insecticide deposit.

The method described below was evolved for the bioassay of treated leaf surfaces, using *Stomoxys calcitrans* as the test insect. It has the advantages that the flies do not have to be anæsthetized or handled, and are forced to walk on the treated surface throughout the period of exposure, irrespective of the duration of this period.

The apparatus used is shown in Fig. 1. It consists of two squares of 'Perspex' (*A* and *B*) measuring 2 × 2 × 3/16 in., each having a central circular hole 1 in. in diameter. The two squares are held together by thin strips of 'Perspex' cemented along opposite edges, but leaving a space to allow a thin 'Perspex' shutter (*C*), 3 × 2 in., to slide between the squares. At one end of the shutter is a hole 1 in. in diameter, so that in one position of the shutter the holes in the three pieces of 'Perspex' are concentric. A 3 × 1-in. glass cylinder (*D*) is cemented into the hole in the 'Perspex' square (*A*).

A 3/16 in. 'Perspex' disk (*E*) cemented to the end of a 4-in. glass rod (*F*) slides smoothly inside the cylinder, through a cork-guide (*G*). The glass rod is fitted with an adjustable rubber ring (*H*) which determines the downward travel of the 'Perspex' disk.

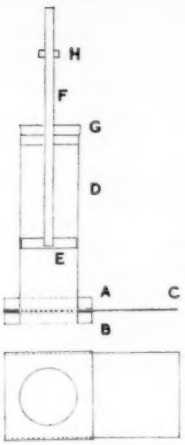


Fig. 1

To use the apparatus, individual flies in 3 × 1-in. tubes are used. A 3 × 1-in. tube containing a single fly is fitted into the hole in 'Perspex' square *B*. The tube containing the fly is darkened by holding in the hand, and the shutter opened, to allow the fly to pass into the apparatus, and the shutter again closed. This operation is performed with the circular disk (*E*) in the fully raised position.

The apparatus is placed on the surface to be treated, the shutter opened to allow access to the treated surface, and the disk depressed to a pre-set position depending on the height of the test insect. Care must be taken to ensure that the fly is in a walking position on the treated surface. In this final position it is impossible for the fly to turn over or walk on the wall of the cylinder.

At the end of the period of exposure, the disk is raised to allow the fly to move off the treated surface, and the shutter then closed. The fly can now easily be transferred to a holding cage.

Other advantages of this method are: (1) With practice, exposures can be performed very quickly, for example, with *Stomoxys calcitrans* it is in the order of the exposure period plus 15 sec. (2) The smooth undersurface of the 'Perspex' disk causes no damage to the dorsal surface of the insect. (3) The apparatus can be dismantled and cleaned by rinsing and swabbing with alcohol. Acetone, benzene and similar solvents cannot be used as they cause crazing of the 'Perspex', and loosening of the cemented joints. This disadvantage would be overcome if the apparatus were made entirely of glass. (4) The apparatus can be made easily and cheaply in any laboratory. (During the preparation of this communication for publication, it was learnt that a similar apparatus had been described by Kernaghan of Northern Nigeria, at a recent meeting of the International Scientific Committee for Trypanosomiasis Research at Jos.)

H. C. M. Parr
J. E. H. Grose

Animal Health Research Centre,
P.O. Box 24,
Entebbe, Uganda.

[1] Wiesmann, R., *Mitt. Schweiz. Ent. Ges.*, **20**, 484 (1947).
[2] Hadaway, A. B., and Barlow, F., *Bull. Ent. Res.*, **40**, 323 (1949); **41**, 603 (1951); **44**, 255 (1953).

GENETICS

Primary Embryonal Sex Ratio in Normal Pregnancies determined by the Nuclear Chromatin

Many attempts have been made to establish a sex ratio for embryos of a younger gestational age. Unfortunately, external genitals are an unreliable index for this purpose before the 5-cm. stage. Former investigations were based on histological examination of gonadal tissue[1]. Recently, Tricomi *et al.*[2] determined the ratio of male to female embryos by examining the placental tissue obtained by curettage for the treatment of incomplete abortions. Their investigation was based on the observations of Graham[3] and Glenister[4], who noted the presence of the sex-chromatin in the trophoblastic cells before gonadal differentiation. Park[5] was able to demonstrate that a morphological sex difference is present in the trophoblastic cells of 10–12-day-old embryos.

The accepted secondary sex ratio of man the world over is 106 males to 100 females. All data available now indicate that more males than females are lost during the early months of pregnancy. Tricomi *et al.*[2] examined 242 abortion specimens and found a ratio of 160 : 100.

The primary sex ratio, that is the ratio which prevails at the time of fertilization, is not yet known. The investigators mentioned accept an incidence of abortions of 10 per cent and by using a weighted mean they assume that the primary sex ratio may approximate 111 : 100, male to female.

Since all investigations until now were carried out on placental tissue obtained from curette material of incomplete abortions, it seemed to us worth while to examine the distribution of sexes in normal early pregnancies.

Fresh placental tissue obtained from 300 medical abortions in apparently normal early pregnancy was investigated. Identification of the sex-chromatin mass was carried out according to the usual histological method. One hundred cells from each specimen were examined at an approximate magnification of 1,000 (oil immersion). The percentage of the sex-chromatin positive cells for female tissue ranged from 30 to 80, that for male from 1 to 8. Preliminary investigations established the reliability of the procedure[6].

165 of our specimens have been classified as male and 135 as female. This result gives a ratio of 122 : 100, male to female. Though the material is too small a series to be conclusive, the results of our investigations indicate a greater incidence of the male sex in the primary ratio than that calculated by Tricomi *et al.*[2].

Since theoretically there should be as many *Y*-bearing as *X*-bearing sperm, the ratio at the time of conception should be 100 : 100. Why an egg should be fertilized more frequently by a sperm with *Y*-chromosome would then have to be explained.

F. E. Szontágh
A. Jakobovits
Ch. Méhes

Department of Obstetrics and Gynæcology,
University Medical School,
Szeged,
Hungary.

[1] Tietze, C., *Human Biol.*, **20**, 156 (1948).
[2] Tricomi, V., Serr, D., and Solish, G., *Amer. J. Obst. Gyn.*, **79**, 504 (1960).
[3] Graham, M. A., *Anat. Rec.*, **119**, 469 (1954).
[4] Glenister, T. W., *Nature*, **177**, 1135 (1956).
[5] Park, W. W., *J. Anat.*, **91**, 369 (1957).
[6] Szontágh, F. E., *Orvosi Hetilap*, **102**, 529 (1961).

A Rare Phenotype within the *Gm* System

In 1956 Grubb[1] published the discovery of a serologically demonstrable γ-globulin factor, which was found to be inherited as a Mendelian dominant. Since then several related factors apparently belonging to a common hereditary system, the *Gm* system, have been demonstrated. At present the system comprises the following factors: *Gm*(*a*) (ref. 1), *Gm*(*x*) (ref. 2), *Gm*(*b*) (refs. 3,4) and *Gm*-like (ref. 5).

Disregarding *Gm*-like, which has not been found in Caucasians, the following phenotypes have been reported: *Gm*($a + x + b +$), *Gm*($a + x - b +$), *Gm*($a + x + b -$), *Gm*($a + x - b -$) and *Gm* ($a - x - b +$). The phenotypes *Gm*($a - x + b +$), *Gm*($a - x + b -$) and *Gm*($a - x - b -$) have not been encountered in spite of rather extensive investigations.

According to the study material factors *Gm*(*a*) and *Gm*(*b*) appear to be Mendelian dominants, being controlled by allelic genes. *Gm*(*x*) also behaves as a Mendelian dominant, but until now has only been demonstrated in sera exhibiting the *Gm*(*a*) factor also. In work involving *Gm*(*a*) and *Gm*(*x*) typing in adults from a series of Danish paternity cases one example of the phenotype *Gm*($a - x +$) was encountered. The serum sample belonged to a healthy woman, A. P., and the result was checked by examining a new blood sample at a later date. The distribution of *Gm*-phenotypes in the series is shown in Table 1.

In order to clarify the significance of the unusual phenotype an investigation of the kindred of A. P. was undertaken. The result of the *Gm* typing is shown in the pedigree (Fig. 1).

It is seen that the quite common phenotypes in the *P*-generation give rise to the rare phenotype

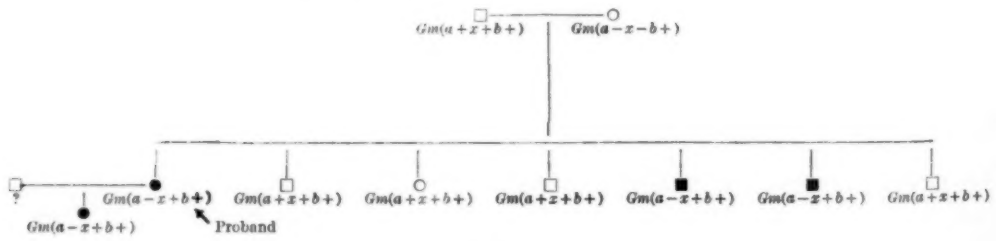


Fig. 1

Table 1

Phenotype	No.	Percentage
$Gm(a+x+)$	201	24·2
$Gm(a+x-)$	272	32·7
$Gm(a-x-)$	358	43·0
$Gm(a-x+)$	1	0·1
Total	832	100·0

$Gm(a - x + b +)$ in 3 out of 7 sibs in the F_1 generation and in the serum of the single representative of the F_2 generation. This may well be explained by the assumption of a rare gene—or closely linked gene combination—*bx*, beside the frequent gene *ax*, the genotype of the father of the proband being *ax*/*bx*.

Our thanks are due to Dr. M. Harboe, Rikshospitalet, Oslo, who checked the results of our *Gm* typing.

K. Henningsen
Jørgen C. Nielsen

Copenhagen University Institute
of Forensic Medicine,
Serological Department,
Frederik 5. vej 9,
Copenhagen.

[1] Grubb, R., *Acta Path. Microbiol. Scand.*, **39**, 195 (1956).
[2] Harboe, M., and Lundevall, J., *Acta Path. Microbiol. Scand.*, **45**, 357 (1959).
[3] Harboe, M., *Nature*, **183**, 1468 (1959).
[4] Harboe, M., *Acta Path. Microbiol. Scand.*, **47**, 191 (1959).
[5] Steinberg, A. G., Giles, B. D., and Stauffer, R., *Amer. J. Hum. Genet.*, **12**, 44 (1960).

Glucose-6-Phosphate Dehydrogenase Activity in Papuans

During a recent investigation into hæmolytic and other anæmias in Papuans the glucose-6-phosphate dehydrogenase (G-6-P D) activity of erythrocytes was estimated in 45 cases. Blood was collected into heparin, and on several occasions into acid–citrate dextrose solution, and dispatched by air to Melbourne, arriving the same day. The G-6-P D activity was estimated in the Biochemistry Department of the Peter MacCallum Clinic, Melbourne, by the method described by Hsia[1]. By this method normal readings are 150–210 units/100 ml. The enzyme appears to be relatively stable for at least a week, provided the cells remain intact. The highest values were obtained in cases of thalassæmia major, acute hæmolytic anæmia of undetermined cause, and congenital hæmolytic anæmia of unknown cause. There was no significant difference in the results obtained, whether A.C.D. solution or heparin was used as an anti-coagulant. The twelve subjects used as controls all gave readings within the accepted range of normality.

Three examples of deficient activity were found. The first was a Papuan adult male, who denied any possibility of racial admixture and appeared to be a typical Papuan from the Rigo district, a few miles from Port Moresby. He was suffering from an attack of hæmolytic anæmia of undetermined cause when first seen on June 12, 1960. Electrophoresis of the hæmoglobin showed no abnormality and the alkaline-resistant hæmoglobin test and the direct Coombs test were both negative. No G-6-P D activity was found at this time. This result was not due to inhibitors, because the addition of blood from a case of thalassæmia major (518 units/100 ml.) gave the expected values. About two months later 8 units/100 ml. of enzyme activity were found and in a further six months the result was 11 units/100 ml. Although the patient was given 30 mgm. of 'Primaquine' daily for 10 days no evidence of hæmolysis was obtained. He stated that his brother had died one year previously from an attack of what seemed to be hæmolytic anæmia. He denied that either of them had eaten beans or taken any drug apart from 'Camoquin' at any time.

The two other patients with low values of G-6-P D were children. One was suffering from thalassæmia major, for which his spleen had been removed. His G-6-P D values on three occasions were 56, 46 and 36 units/100 ml. The other child was suffering from anæmia apparently due to tuberculosis infection and the G-6-P D value was found to be 66 units/100 ml. It has not been possible to confirm this result.

It is probable that anæmia which is determined by genetical factors is not nearly as common in New Guinea as is anæmia due to parasitic and nutritional factors. However, the finding of thalassæmia[2] and deficiency in G-6-P D in the Territory suggests that further attention should be given to investigating genetical factors.

B. P. K. Ryan
Port Moresby General Hospital,
Papua.

I. C. Parsons
Peter MacCallum Clinic,
Melbourne.

[1] Hsia, D. V., *Inborn Errors of Metabolism*, 331 (Year Book Publishers, Chicago, 1959).
[2] Ryan, B. P. K., *Med. J. of Australia* (in the press).

VIROLOGY

Cyclic Events in the Viral Cycle

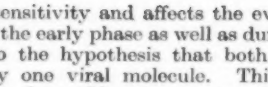
The polio virus is able to develop only within given limits of temperature. The simplest reason for this seems to be that low and high temperatures block viral development by altering the metabolism of the host cell. Yet mutants of the polio virus can be obtained which thrive at these low or high temperatures at which the original type is unable to multiply: the sensitivity of viral development to temperature is thus controlled by the genetic constitution of the virus. Moreover, it was discovered that one mutational event shifts the whole curve expressing the sensitivity of viral development to temperature towards the left or the right: psychrosensitivity decreases when thermosensitivity increases and vice versa[1].

The polio virus is able to develop normally if submitted to supra-optimal temperatures during the first part of the cycle only or to infra-optimal temperatures during the second part only. Infra-optimal temperatures, which do not block the second part, interfere with the primary phase whereas supra-optimal temperatures, which do not block the first part, interfere with the secondary phase. The polio virus is, in fact, able to complete its development at high and low temperatures both incompatible with viral multiplication, provided they alternate in the right sequence during one growth-cycle.

The effects of infra-optimal temperatures are increased by heavy water, the effects of supra-optimal temperatures decreased. In the presence of heavy water the whole curve expressing the sensitivity of viral development to temperature is shifted: heavy water decreases the apparent temperature[2].

The fact that a single mutation modifies at the same time in opposite directions the psychro- and

the thermo-sensitivity and affects the events taking place during the early phase as well as during the late one, leads to the hypothesis that both phases are controlled by one viral molecule. Things happen as if this molecule would undergo reversible structural alterations, the equilibrium of the reaction being shifted by temperature changes.



The state A would allow the completion of the primary phase only, the state B the completion of the secondary phase only. Infra-optimal temperatures, by shifting the probability of the molecular structure towards B, prevent the completion of the early phase. Supra-optimal temperatures, by shifting the probability of the molecular state towards A, prevent the completion of the secondary phase. The effect of deuteration shows that hydrogen bonds are involved in the reaction $A \rightleftharpoons B$.

The optimal temperature for viral development probably corresponds to the transition temperature of the reaction at which both states of the alleged viral molecule are equally represented. Infra- and supra-optimal temperatures decrease or block viral development by shifting the equilibrium, thus favouring one of the states at the expense of the other. The completion of the viral cycle would thus imply a reversible cyclic alteration of a metastable molecule[3].

During the life-cycle of a cell a certain number of molecules, or molecular complexes, such as mitotic fibres, genes, chromosomes, undergo reversible alterations of structure. On the other hand, vegetative functions, that is, the activity and the biosynthesis of enzymes, are regulated by feed-back mechanisms depending in the last analysis on structural molecular changes. It is felt that reproductive and 'cyclic' functions must also be under the control of some feed-back regulating mechanisms. Metastable molecules able to undergo reversible structural alterations could be responsible for these mechanisms[4].

This work was supported by a grant from the U.S. National Foundation.

André Lwoff
Marguerite Lwoff

Institut Pasteur,
Paris.

[1] Lwoff, A., and Lwoff, M., *Ann. Inst. Pasteur*, **98**, 173 (1960). Groman, N., Lwoff, A., and Lwoff, M., *ibid.*, **98**, 351 (1960).
[2] Lwoff, A., and Lwoff, M., *C.R. Acad. Sci., Paris*, **251**, 3131 (1960); **252**, 223 (1961).
[3] Lwoff, A., and Lwoff, M., *Ann. Inst. Pasteur*, **101**, 469, 478, 490 (1961).
[4] Lwoff, A., and Lwoff, M., *J. Theor. Biol.* (submitted for publication).

Multiplication of Mouse-adapted Influenza Virus in Mouse Lungs after Infection with Very Low Doses

We studied the dynamics of the infection with influenza virus in mice after intra-nasal administration of extremely low doses. White mice were infected with the mouse-adapted Em_2 strain of the $A1$ type influenza virus. The virus, used in the form of infected allantoic fluid, showed LD_{50} titres of $10^{5.1}$ and $10^{5.45}$ 0·1 ml., respectively, as estimated by intra-nasal inoculation of white mice, and a $TCID_{50}$ titre of $10^{4.88}$ and $10^{5.52}$ 0·1 ml., respectively, as determined in suspended chlorio-allantoic membrane tissue cultures[1]—herein designated as Horváth's *TC*. Mice weighing 16–18 gm. were inoculated under light ether anæsthesia intra-nasally in individual experiments (altogether 6 experiments in 3 independent series) with 0·05 ml. of infected allantoic fluid, containing $10^{-2.12}$–$10^{0.88}$ $TCID_{50}$ 0·1 ml.; in other words, in each case with a lower dose than 1 mouse LD_{50}. Starting with the first day after inoculation, 3 mice were killed daily and from their lungs 10 per cent suspensions in Horváth's[1] nutrient medium were prepared. The suspensions were clarified by centrifugation at 2,500 r.p.m. for 10 min. and serial 10-fold dilutions of them inoculated into Horváth's *TC*. The cultures were incubated at 35° C. for 72 hr. and the reproduced virus detected by hæmagglutination test on plastic plates[2]. The results are shown in Fig. 1. It can be seen that the multiplication of the virus in the lungs depends on the dose of the virus administered and shows a cyclic character. After administration of the lowest dose (0·0038 $TCID_{50}$ in 0·05 ml.), virus was first detected three days after infection. It is still present on the fourth day, but thereafter disappears and is not detectable up to day 12. After administration of a 10-times higher dose the virus appears in the lung also on day 3, it is not detectable for the next five days and appears repeatedly on day 9 or 10 after infection. The higher the dose administered, the shorter the time intervals

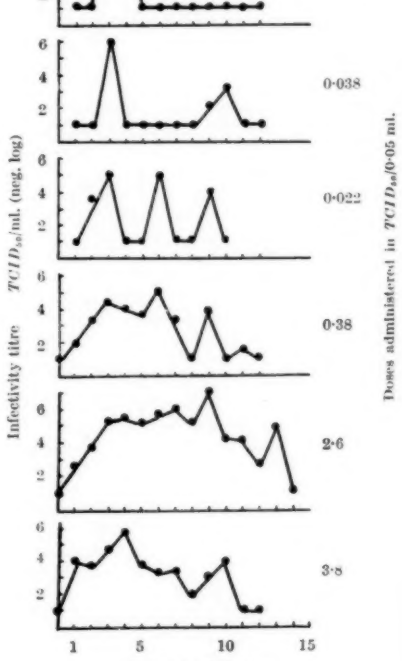


Fig. 1

of the cyclic appearance and disappearance of virus in the lungs. After infection with 2·6 $TCID_{50}$ and 3·8 $TCID_{50}$ in 0·05 ml., respectively, a complete disappearance of virus from lungs was not detected during the whole observation period, although even in these cases an appreciable variation in infectivity titres was seen. It is to be noted that the first disappearance of virus and/or a marked lowering of its titre occurred in all experiments 4–5 days after infection. Virus-neutralizing antibodies were not detected in sera of mice for 10–14 days after infection.

We supposed that the disappearance or the marked drop in the infectivity titre of the virus in mouse lungs was due to an interferon-like substance, formed as a consequence of the virus infection. Therefore, 0·1 ml. of undiluted suspensions, in which no virus could be detected, were inoculated into Horváth's *TC* and incubated at 35° C. for 24 hr. Thereafter the cultures were treated with a heterologous influenza virus (*A*; *PR8*). We found that after 72 hr. incubation the infectivity titre of the added virus was lowered—in comparison with controls—by 90 per cent. Virus-containing lung suspensions were tested for the presence of an interferon-like substance. The suspensions were kept at 4° C. for six weeks. During storage the virus was inactivated, for no virus reproduction could be detected after inoculation of such suspensions into Horváth's *TC*. Then Horváth's *TC* was inoculated with these suspensions and treated with homologous (Em_2) and heterologous (*PR8*) virus, respectively, in the same way as mentioned above. In these cases also 90–100 per cent decrease in infectivity titres—in comparison with the controls—were observed. Lung suspensions of non-infected mice, to which Em_2 virus was added *in vitro* and stored also for six weeks at 4° C., did not inhibit the reproduction of the added virus at all. It must be noted that also in these control lung suspensions the virus was inactivated completely during storage. We designate the substance formed in mouse lungs on infection with influenza virus and inhibiting the multiplication of added virus as an 'interferon-like substance', although it is very probable that the interference phenomenon observed in our experiments was caused by Isaacs's[3] interferon, taking into account the results published by Isaacs and Hitchcock[4].

The observed cyclic reproduction of influenza virus in mouse lungs is in agreement with Wagner's[5] hypothesis concerning the interaction of virus and interferon in permanently infected tissues. We suggest that when non-lethal doses are used on mice, the interferon produced prevents the aggressive spread of infection by occupying most of the susceptible cells in the vicinity of the primary focus of the infection. The possibility of a similar interaction of virus and interferon should be assumed after infection of human beings with influenza virus. Interferon could prevent, in some cases, the manifestation of the infection until specific antibodies are formed.

F. Link
J. Raus

Institute of Virology,
Czechoslovak Academy of Sciences,
Bratislava, 9.

[1] Horváth, I., *Acta Microbiol. Hung.*, **1**, 481 (1954).
[2] Takátsy, Gy., *Acta Microbiol. Hung.*, **3**, 191 (1955).
[3] Isaacs, A., and Lindenmann, J., *Proc. Roy. Soc.*, B, **147**, 258 (1957).
[4] Isaacs, A., and Hitchcock, G., *Lancet*, ii, 69 (1960).
[5] Wagner, R. R., *Bact. Revs.*, 24, (1960).

AGRICULTURE

Nitrogen-fixing Blue-Green Algæ in Australian Rice Soils

A FEATURE of myxophycean algæ is the capacity of certain members of the class to fix elemental nitrogen. Blue-green algæ are widely distributed in Nature. They are known to be particularly abundant in regions devoted to the cultivation of rice where the activities of nitrogen-fixing species probably make significant contributions to the maintenance of soil fertility[1,2]. Australia offers excellent opportunities for research. Previously, these have not been exploited even though 46,000 acres are sown to rice annually in the temperate Murrumbidgee Irrigation Area and extensive tracts of country are being developed for rice production in tropical Northern Australia.

As a first step, I have undertaken a survey to determine the distribution of nitrogen-fixing blue-green algæ in the rice areas of Australia and to build up a culture collection for use in laboratory work.

During a visit to Darwin in February 1960, a number of samples was collected from the developing commercial rice crop at Humpty Doo, from the experimental plots of the Commonwealth Scientific and Industrial Research Organization in the same area, and from the two Northern Territory Administration experimental rice stations at Beatrice Hill and Marrakai. Humpty Doo and Beatrice Hill are on the heavy alluvial clays of the Adelaide River and Marrakai is on the somewhat lighter soils of the 'bull dust' plains[3].

The samples were collected in sterile bottles in various situations including supply ditches, at the water surface in the rice crops, from the rice plants and from the submerged soil surface. Every effort was made to ensure that the material was representative of the areas surveyed. No more than five days elapsed between collection of the samples and their return to the University of Sydney.

It is of interest to note that, while blue-green algæ apparently capable of nitrogen-fixation were isolated from almost every sample, their presence in the field was not always obvious. Although typically gelatinous colonies of *Nostoc* were found sometimes to be abundant in the rice fields, they were not common. At present, no simple explanation can be offered for their uneven distribution. In general, algal development was most marked where the movement of the irrigation water was reduced to a minimum and its turbidity was low. Nitrogen-fixing species were not found in the experimental plots possibly because the level of soil nitrogen had been raised considerably by fertilizer applications.

Uni-algal cultures were obtained by routine culture techniques using a range of media both in the liquid form and solidified with agar. Those capable of continued growth on a nitrogen-deficient medium were retained. The composition of this medium, a Winogradsky nutrient solution modified by Acierto (personal communication) and known as No. 5, is: KH_2PO_4, 0·25 gm.; $MgSO_4.7H_2O$, 0·125 gm.; NaCl, 0·125 gm.; $FeSO_4.7H_2O$, 2·5 mgm.; $MnSO_4.4H_2O$, 2·5 mgm.; $NaMoO_4.2H_2O$, 0·25 mgm.; $CaCO_3$, 5·5 gm.; double glass-distilled water, 1 l.; *p*H 7·2–7·4.

The Northern Territory collection comprises some 36 cultures, none of which has been identified fully.

Only two genera are represented, *Nostoc* and *Anabaena*, although one isolate may be a species of *Cylindrospermum*. Four isolates have been obtained in a bacteria-free state. In December 1960, a survey was conducted in the Murrumbidgee Irrigation Area of New South Wales. The samples collected are being treated in the same manner as those from the Northern Territory. Already one culture, a species of *Anabaena*, has been obtained free of contaminants.

Algæ with probable nitrogen-fixing capacities were found to be a minor element of the algal flora under these conditions. However, species of *Anabaena* were almost ubiquitous. *Nostoc* was not widespread and representatives of other genera, reported to be nitrogen-fixing, were not found. It should be noted that heavy dressings of ammonium sulphate are applied to the soils and that copper sulphate is introduced commonly into the irrigation water in concentrations up to 5 p.p.m. This practice has been adopted, in fact, to control the growth of algal 'scum', largely *Spirogyra*, *Lyngbya* and *Phormidium*, which interferes significantly with the early growth of the rice seedlings.

Gelatinous colonies, closely resembling those of a *Nostoc* found in the Northern Territory, were found only occasionally floating in supply ditches and in the rice fields. Subsequent examination has shown these algæ to be a species of *Gloeotrichia*. It will be of interest to determine whether this organism is capable of nitrogen fixation. The Kimberley region of Western Australia, where rice is also being grown, has not yet been surveyed. However, several soil samples from that area have been examined. It is not regarded as significant that nitrogen-fixing algæ could not be found in this material. Attempts are at present being made here to study some of the factors affecting the growth and fixation of nitrogen by several of the bacteria-free isolates in the collection.

Financial support for this work is being provided by a research grant from the University of Sydney. During the survey, assistance was given by the personnel and officers of both private and government bodies. I am grateful to them.

J. S. Bunt

Microbiology Laboratories,
School of Agriculture,
University of Sydney.

[1] De, P. K., *Proc. Roy. Soc.*, B, **127**, 121 (1939).
[2] Watanabe, A., Nishigaki, S., and Konishi, C., *Nature*, **168**, 748 (1951).
[3] *Soils of the Katherine Darwin Region, Northern Territory*, Commonwealth Scientific and Industrial Research Organization Soil Publication No. 6, Melbourne (1950).

Control of the Army Worm on Grazing in Kenya

Infestations of *Laphygma exempta* Wlk. (Lep. Noctuidae) on natural grazing present a problem in control because conventional tractor or aerial spraying is uneconomical, and aerial drift spraying is impractical on many farms due to their small area and irregular shape, and to the localization of cleared paddocks between strips of uncleared vegetation. Such infestations have therefore been considered uncontrollable and have been left to complete their cycle with a consequent severe loss of grazing. The development of the exhaust sprayer for the control of *Schistocerca gregaria* (Forsk)[1,2] offered a cheap and speedy method of control and six tests with this appliance were performed by us on an outbreak in 1959.

The sprayer was mounted on a four-wheel-drive vehicle fitted with a governor control which enabled it to be driven at a speed of 5 m.p.h., and to give an emission-rate of 3–4 gall. of spray material a mile. The spray nozzle was mounted 6 ft. above the ground and the spray plume was emitted vertically upwards so that, with the vehicle travelling across wind, a drift of 100–150 yd. was obtained in quite light breezes. A standard formulation containing 20 per cent dieldrin in heavy oil was used in the first test, but thereafter crude formulations of DDT in light oil mixed 1 : 1 with engine oil were used in order to reduce toxicity hazards to operators and to stock.

The results of the tests are summarized in Table 1 together with data on air movement and vegetation. Assessments have been recorded visually because this was the only method used in tests 1 and 2, while in 4 a post spraying count was obviously unnecessary, and in 5 and 6 reductions of a fairly high order had occurred on the controls. In test 3, a pre-spraying count gave an estimate of four larvæ/ft.^{2} while 72 hr. after spraying the estimated populations (based on sixty samples of one foot square from each plot in an area between 7 and 40 yd. downwind of the spray track) were:

Treatment	Larvæ/ft.^{2}
Control	3·78 ± 0·58
Single application	1·37 ± 0·20
Double application	0·87 ± 0·20

The post spraying counts were intentionally biased on the treated plots, samples being taken in the thickest grass cover where initially more larvæ would have congregated, but even ignoring this bias, the reduction is very satisfactory.

The failure in test 4 may be attributed to a combination of factors. The very slight air movement was

Table 1

No.	Insecticide (per cent)	Wind	Vegetation	Result after 72 hr.	
				Sprayed area	Control
1	Dieldrin 20	Light	Grass, occasional trees	Heavy reduction	Slight reduction
2	DDT 12·5	*c.* 5 m.p.h.	Good grass cover, no bushes	Heavy reduction	No change
3	DDT 12·5	*c.* 5 m.p.h.	Good grass cover, no bushes	Heavy reduction	No change
	DDT 12·5 × 2*	*c.* 5 m.p.h.	Good grass cover, no bushes	Very heavy reduction	No change
4	DDT 12·5	Negligible	Very thick grass cover, numerous tall trees	No change	No change
5	DDT 15·0	*c.* 5 m.p.h.	Sparse grass, some bushes	Very heavy reduction	Rather heavy reduction
6	DDT 15·0	Light	Sparse grass, no bushes	Heavy reduction with numerous dead larvæ	Heavy reduction; no dead larvæ

* Two runs in opposite directions were made along the same track in order to double the rate of application.

severely interrupted by the numerous trees so that dispersion of the spray was unsatisfactory. Secondly, the heavy grass cover, in places 9 in. deep, caused dilution of the spray in terms of quantity per unit volume of vegetation.

In test 5, the results were confused by a nuclear polyhedrosis which caused a pronounced reduction on the controls. Although the decrease was greater on the treated area, the spray was obviously applied to a debilitated population and had only enhanced the action of the disease.

Test 6 was difficult to assess, as heavy reductions had occurred on both treated and untreated areas. However, on the control, the reduction was due entirely to pupation and no dead larvæ could be found on the surface; on the treated area, although pupation may have had some effect, many dead larvæ were found on the surface. The treatment may therefore be regarded as having caused a diminution in numbers.

The distribution of live larvæ after treatment accorded well with the observed distribution of spray recorded by Courshee[3]. In tests 2, 3 and 5, no deaths due to spraying occurred within 5 yd. of the spray track; mortality then increased to a peak at 30–50 yd. where very few live larvæ could be found, and beyond this limit decreased more slowly to become negligible 100–110 yd. from the spray track.

Should further tests fulfil the promise of those conducted, we believe the exhaust sprayer will have great value in the control of *L. exempta* and other 'army worms' on grazing. Its use is limited by the nature of the vegetation only in that the presence of too many trees interrupts air movement and too thick a grass cover causes excessive dilution. Its value lies in its low capital cost, low running costs, speed, and manœuvrability.

We envisage two uses for the sprayer: (1) it can be used to provide a barrier between infested and uninfested grazing along a wide front; (2) it can be used to eliminate infestations from paddocks by parallel spray runs at intervals of 75–100 yd. In this latter case, using a cost of 20*s.*/gall., a rate of application of 4 gall./mile, and a distance between runs of 75 yd., the spray would cost 3*s.*/acre plus the running costs of the vehicle.

We are grateful to the Director of the Desert Locust Survey, Nairobi, for the loan of the exhaust sprayer, and for the advice given by him and his staff on its use.

J. A. Bullock
D. C. Bond

Entomology Section,
Department of Agriculture,
Kenya.

[1] Sayers, J. H., and Rainey, R. C., Desert Locust Survey (1958).
[2] Sayers, J. H., *Bull. Ent. Res.*, **50**, 371 (1959).
[3] Courshee, R. J., *Bull. Ent. Res.*, **50**, 355 (1959).

SOIL SCIENCE

Influence of Some Organic Salts on the Absorption of Potassium by Vermiculite

A marked effect of the ammonium ion on release and fixation of potassium by vermiculite has been shown by many workers. The presence of ammonium ions in an extracting solution inhibits the removal of potassium which has been absorbed or fixed in vermiculite. The fixation of potassium by vermiculite is also reduced if the solution contains ammonium ions. The effect of the ammonium ion on potassium reactions is presumably due to the similarity of the two ions with respect to their size and chemical reactivity. Both ions are known to have an optimum ionic diameter for fixation within the vermiculite lattice. In the presence of ammonium ions the vermiculite lattice apparently is contracted so that potassium ions can neither enter nor leave the internal vermiculite surfaces.

The influence of other nitrogen compounds on potassium absorption by vermiculite was considered. The term 'absorption' is used here in preference to 'adsorption' since most of the effects noted here are associated with the internal surfaces of the vermiculite mineral. The compounds used in this work were amines of various kinds which may be considered in a sense as analogues of the ammonium ion in that one or more of the hydrogen atoms around the nitrogen are replaced with organic groups of various kinds. The substitution of organic groups for the hydrogens of the ammonium ion of course results in great changes in the properties of the ion.

Two types of investigations were made. One was concerned with the effect of the organic salts on the rate of potassium absorption by vermiculite, the other with the effect of organic salts on the amount of absorption under conditions where equilibrium could be approached. The vermiculite used in this work had a cation exchange capacity of 160 m.equiv./100 gm.

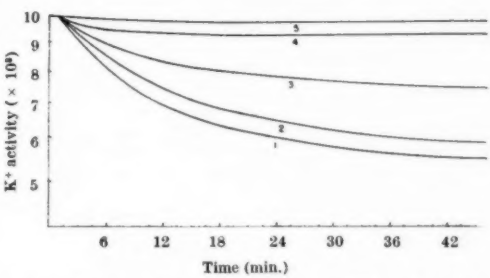


Fig. 1. Potassium activity in the presence of 0·25 gm. of 150–250μ diameter vermiculite as a function of time at 20° C. 1, No other salt; 2, 1 m.equiv. of *n*-butylamine hydrochloride; 3, 0·1 m.equiv. 2,4-diaminophenol dihydrochloride; 4, 1 m.equiv. aniline hydrochloride; 5, 1 m.equiv. 2,4-diaminophenol dihydrochloride

Fig. 1 shows the effect of some organic salts on the activity of potassium in the presence of vermiculite as a function of time. The activity measurements were made continuously with the 78137 *V* cationic electrode produced by Beckman Instruments, Inc., in combination with a Beckman Zeromatic *p*H meter and a Sargent recorder. The potassium chloride solution was stirred continuously with a magnetic stirrer. After the recorder deflexion was static, the organic salt was added. There was only a small effect of the organic salts on the potassium ion activity since the amount added was not enough to affect the activity coefficient of the potassium ion a great deal. Also the electrode was insensitive to the organic ions used although it was found to be sensitive to the presence of ammonium ions. At zero time 0·125 gm. of vermiculite was added. A short lag time of about 1 min. is apparent in Fig. 1 before the potassium started disappearing from solution. This lag time was noted

Table 1. 001 Spacings of Vermiculite after Contact with Potassium Chloride in the Presence of Various Salts

Compounds	M.equiv. of compound present	Vermiculite (gm.)	M.equiv. of KCl present	Hr. contact	001 spacing (Å.)
None	—	0·25	0·5	2	12·1 and 10
2,4-Diaminophenol dihydrochloride	1·0	0·25	0·5	2	14·5
2,4-Diaminophenol dihydrochloride	0·1	0·25	0·5	2	14·3 and 12·6
Aniline hydrochloride	1·0	0·25	0·5	2	14·5
n-Butylamine hydrochloride	2·0	0·25	0·5	2	12·1 and 10
n-Butylamine hydrochloride	1·0	0·25	0·5	2	12·1 and 10
n-Butylamine hydrochloride	0·1	0·25	0·5	2	12·1 and 10
p-Phenylene diamine dihydrochloride	0·25	0·125	0·25	48	14·5–13·0
Histidine hydrochloride	0·25	0·125	0·25	48	13·8–12·3
Ammonium chloride	0·25	0·125	0·25	48	11·3 and 10
Lysine hydrochloride	0·25	0·125	0·25	48	11·3 and 10

in all cases and is probably the time required for the complete wetting of the mineral. The magnesium ion was initially the saturating cation on the exchange complex of the vermiculite, the electrode being insensitive to this ion.

The main observation to be made is that *n*-butylamine hydrochloride (curve 2) had little effect on the rate of absorption of potassium as compared with the rate when no organic salt was present (curve 1). On the other hand, 2,4-diaminophenol dihydrochloride (curves 3 and 5) and aniline hydrochloride (curve 4) greatly affected potassium absorption. The 2,4-diaminophenol dihydrochloride added at the 0·1 m.equiv.-level (curve 3) reduced absorption of potassium vermiculite much more than did 1·0 m.equiv. of *n*-butylamine hydrochloride (curve 2). The 001 spacings of vermiculite obtained by X-ray diffraction 2 hr. after the absorption reactions began are given in Table 1.

Table 2 shows the amounts of potassium absorbed from solution by sodium-saturated vermiculite in the presence of various organic salts, ammonium chloride, and in the instance when no foreign salts were present. These analyses were made on the solution phase with a Coleman flame photometer after 48 hr. were allowed for absorption. Large differences are apparent in the effects of organic salts on potassium absorption. Particularly noteworthy is the fact that the *n*-butylamine hydrochloride had no effect whatsoever on potassium absorption at either of the two concentrations. Also, as noted in Table 1, the 001 spacing of the lattice has contracted in exactly the same manner as where only potassium chloride was present. On the other hand, some of the amines, particularly those with a cyclic molecular structure, greatly inhibited the absorption of potassium. Apparently the number of electrical charges per molecule was not too important since the aniline hydrochloride was about as effective as 2,4-diaminophenol dihydrochloride and para-phenylenediamine dihydrochloride in preventing potassium absorption. Of the three amino-acid salts examined, histidine monohydrochloride was the most effective in inhibiting absorption. Histidine possesses a ring structure while the other two amino-acids used are linear in form. Histidine was almost as effective as the ammonium ion in preventing potassium absorption.

Table 2. Absorption of Potassium by Vermiculite in the Presence of Some Salts*

Salt	M.equiv. K absorbed/100 gm. Salt/KCl ratio 0·1	1·0
n-Butylamine hydrochloride	120	120
2,4-Diaminophenol dihydrochloride	108	36
Aniline hydrochloride	106	32
p-Phenylenediamine dihydrochloride	108	28
Tetramethylammonium chloride	118	100
Histidine monohydrochloride	112	58
Arginine monohydrochloride	108	96
Lysine monohydrochloride	120	88
Ammonium chloride	106	52
None	120 m.equiv. K^+/100 gm. absorbed when no other salt was present	

* Absorption by 0·125 gm. vermiculite in 200 ml. of $1·25 \times 10^{-3}$ *N* KCl after 48 hr. at 20° C. Each figure is the average of analyses of duplicate samples with extremely low variation between duplicates.

Apparently the organic molecules which affect potassium absorption do so in a manner different from that of the ammonium ion. Table 1 shows that the 001 spacing of the vermiculite collapsed to spacings of 11·3 and 10 Å. in the presence of ammonium chloride. However, the lattice remained pretty well expanded in the presence of the organic molecules which effectively prevented absorption. Those organic molecules which did not greatly affect potassium absorption allowed a greater amount of potassium to enter the internal surfaces. This resulted in a greater degree of contraction of the lattice as shown by the smaller values of the 001 spacings. The potassium thus entrapped in the internal surfaces of the contracted lattice of the vermiculite was fixed and was not exchangeable under ordinary conditions of cation exchange.

Since the vermiculite lattice was fully expanded in the presence of those organic cations which most effectively prevented potassium absorption, these ions undoubtedly prevent the entrance of potassium ions into the internal surfaces of the vermiculite structure. This could be accomplished if the energy of adsorption of the organic ion were so large that the potassium from the solution phase could not exchange with the organic cation at the edges of the mineral. The potassium thus would not be able to move into the interlayer spaces. Undoubtedly the mechanism of cation movement into the lattice is diffusion along cation exchange sites[1]. In order for this to occur, the potassium must be able to exchange with other ions on the cation exchange complex. If the cation exchange complex were 'clogged' with ions which potassium could not displace, the potassium would be unable to enter the internal vermiculite surfaces.

In soils, a large number of different kinds of organic molecules appear as the result of the decomposition of organic matter. Some of the organic molecules used in this work were more effective than the ammonium ion in preventing potassium absorption, while histidine, a possible product of decomposition of organic matter, was almost as effective as the ammonium ion. Organic molecules may play an important part in the release and fixation of potassium by vermiculitic clays in soils. The complete lack of effect of the *n*-butylamine ion on potassium absorption was anomalous since all the other compounds used in these experiments had some effect. This amine apparently did not compete with the potassium for cation exchange sites.

The apparent interaction of organic compounds in potassium reactions with vermiculite may be of importance in soils containing this mineral. Fixation, and possibly release of potassium, may be modified by the presence of certain organic compounds.

M. M. Mortland
Michigan Agricultural Experiment Station,
East Lansing, Michigan.

[1] Walker, G. F., *Nature*, **184**, 1392 (1959).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 6

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. M. A. Floyer: "Possible Factors in the Mechanism of Hypertension".*

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. W. H. Thorpe: "The Idea of Emergence in Biology".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. G. Medhurst: "The General Problems of F.M. Multi-Channel Communications".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. J. Meixner (Aachen): "On the Theory of Linear Passive Systems".* (Further lectures on November 7 and 9.)

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. N. Goodway: "The U.K.A.E.A. and its Relations with Industry".

SOCIETY OF INSTRUMENT TECHNOLOGY, CONTROL SECTION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. P. R. Wyman: "Semiconductor Diodes and Rectifiers in Control Engineering"; Dr. G. D. Bergman: "The Transistor in Control Engineering".

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the WOOLWICH POLYTECHNIC SCIENTIFIC SOCIETY, at the Woolwich Polytechnic, Thomas Street, London, S.E.18), at 7.30 p.m.—Prof. C. W. Davies: "Ion Exchange Resins".

Tuesday, November 7

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Prof. G. C. Allen: "The Impediments to Britain's Industrial Progress".*

SULGRAVE MANOR BOARD (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5 p.m.—Mr. Stephen Toulmin: "Science and the National Mind". (Further lectures on November 9, 14 and 16.)

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. H. E. Huxley: "Structural Aspects of Muscular Contraction".* (Seventh of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICATION AND WEAR GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Refrigeration Lubrication".

UNIVERSITY OF LONDON (in the Library of the Institute of Bankers, 10 Lombard Street, London, E.C.), at 6 p.m.—Prof. D. T. Jack: "Industrial Relations".* (Second of five lectures on "Britain's Economic Future" organized by the Department of Extra-Mural Studies and the Institute of Bankers.)

SCIENCE FORUM (at Friends House, Euston Road, London, N.W.1), at 7.30 p.m.—Prof. L. S. Penrose, F.R.S.: "Genetics, Radiation and the Future".

Wednesday, November 8

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. C. P. Stewart: "Acid-base Metabolism in Modern Terms".*

BRITISH INSTITUTION OF RADIO ENGINEERS, MEDICAL ELECTRONICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. E. L. Gregory and Mr. E. A. Piper: "Electronics in Chromatography".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Dr. H. E. Merritt: "Gear-Tooth Contact Phenomena".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Dr. H. E. C. Powers: "Crystallization of Sucrose".

BRITISH INSTITUTE OF MANAGEMENT (at the Connaught Rooms, London, W.C.2), at 6.30 p.m.—Field-Marshal the Viscount Slim, K.G.: "Leadership" (Seventh Elbourne Memorial Lecture).

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMISTRY GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "Derivatography as a New Thermoanalytical Method" introduced by Prof. L. Erdey (Budapest University).

WOMEN'S ENGINEERING SOCIETY (at "Hope House", 45 Great Peter Street, Westminster, London, S.W.1), at 7 p.m.—Mr. R. Walles: "Windmills—Their Rise and Decline".

Wednesday, November 8—Thursday, November 9

ZOOLOGICAL SOCIETY OF LONDON, and the ASSOCIATION FOR THE STUDY OF ANIMAL BEHAVIOUR (in the Meeting Room of the Zoological Society of London, Regent's Park, London, N.W.1), at 10.30 a.m. daily—Eighth Symposium.

Wednesday, November 8

"Evolutionary Aspects of Animal Communication".

Thursday, November 9

"Imprinting and Early Learning".

Wednesday, November 8—Friday, November 10

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2)—Conference on "Non-destructive Testing in Electrical Engineering".

Thursday, November 9

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. H. Judith Milledge: "Form and Colour in Nature".*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. M. J. Lighthill, F.R.S.: "Sound Generated Aerodynamically" (the Bakerian Lecture).

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. Wm. T. Stearn: "Some Puzzling Linnean Abbreviations"; Mr. P. R. O. Bally: "The Retreat of Nature in Modern East Africa".

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Dr. Harold Heywood: "New Developments in the Use of Solar Energy".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. J. Meixner: "On the Theory of Linear Passive Systems".* (Last of three Special University Lectures.)

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Brains Trust on "Automatic Control" (Inaugural Meeting).

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. R. S. Teeble: "Applications of Microwave Spectroscopy".

RADAR AND ELECTRONICS ASSOCIATION (in the Lecture Hall of the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7 p.m.—Dr. W. F. Hilton: "Space Communications. Part 2: Orbits and their Economics".

ROYAL INSTITUTE OF CHEMISTRY (at Croydon Technical College, Fairfield, Croydon, Surrey), at 7.30 p.m.—Mr. R. B. Collins: "Modern Colour Photographic Processes".

Friday, November 10

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. F. Fenner (Australian National University): "Myxomatosis—a Study of Evolutionary Changes in an Infectious Disease".*

UNIVERSITY OF LONDON (at the Royal Holloway College, Englefield Green, Surrey), at 8.15 p.m.—Mr. William Cooper: "Science and Literature".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. W. S. Bullough: "Modern Research on the Skin of Mice and Men".

Saturday, November 11

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. E. J. Lindgren: "Reindeer Farming in Europe, Asia, and North America".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LIBRARIAN (graduate with some library experience)—The Secretary and Registrar, The University, Highfield, Southampton (November 11).

LECTURER or ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, The University, Sheffield (November 11).

LECTURER or ASSISTANT LECTURER (preferably a specialist in the field of human geography with first-hand experience of a major overseas area outside Europe) IN GEOGRAPHY—The Registrar, The University, Leeds 2 (November 13).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (honours graduate with appropriate training and research experience), to work as a member of a team undertaking research on hill sheep nutrition—The Secretary, Hill Farming Research Organisation, 48 Palmerston Place, Edinburgh 12 (November 13).

LECTURER in EXPERIMENTAL PHARMACOLOGY—The Secretary of University Court, The University, Glasgow (November 15).

LECTURER IN PSYCHOLOGY at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 15).

ASSISTANT LECTURER or LECTURER IN ANIMAL HUSBANDRY—The Registrar, The University, Liverpool, quoting Ref. CV/T (November 18).

LECTURER IN CYTOLOGY IN THE DEPARTMENTS OF BOTANY AND ZOOLOGY in the Faculty of Science—The Registrar, The University, Manchester 13 (November 18).

LECTURER or ASSISTANT LECTURER IN STATISTICS—The Registrar, The University, Sheffield (November 18).

SCIENTIFIC ASSISTANT (with at least G.C.E. at "O" level in four subjects, including geography, with an interest in biology and a capacity for neatness and precision), for recording and mapping reported movements of locust swarms, copying-maps, and generally assisting in the geographical work of a small team—The Director, Anti-Locust Research Centre, 1 Princes Gate, London, S.W.7 (November 18).

LECTURER (with an interest in electronics or telecommunications) IN ELECTRICAL ENGINEERING at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 29).

CHAIR OF PHILOSOPHY IN THE SCHOOL OF GENERAL STUDIES, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

LECTURERS IN THE DEPARTMENT OF PHYSICS, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 30).

PROFESSOR OF EXPERIMENTAL PHYSICS—The Registrar, The University, Nottingham (November 30).

READER or SENIOR LECTURER IN ARCHÆOLOGY at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 30).

LECTURER IN SEDIMENTARY PETROLOGY, to initiate and direct research on sedimentation in Cardigan Bay and St. George's Channel, as part of an investigation into the origin and geological history of this region—The Registrar, University College of Wales, Aberystwyth (December 15).

LECTURER or ASSISTANT LECTURER IN THE HISTORY OF SCIENCE at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 31).

AGRICULTURAL OFFICERS (male, with a degree in agriculture or horticulture and at least two years postgraduate experience) IN THE DEPARTMENT OF AGRICULTURE, Tanganyika, for extension work among both African and non-African farmers and experimental work on agricultural stations—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting RC.213/145/01/C1.

ASSISTANT CHEMIST (male or female, with H.N.C. in chemistry or biochemistry or an equivalent qualification), for routine and development work—The Secretary, Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, W.2.

BIOCHEMIST (Basic grade) IN THE DEPARTMENT OF ENDOCRINOLOGY—The Hospital Secretary, New End Hospital, London, N.W.3.

COCOA OFFICER (male, under 45, with a degree in agriculture, preferably in cocoa extension or development work) IN THE DEPARTMENT OF AGRICULTURE, British Solomon Islands Protectorate, to assist in cocoa development and extension of cocoa planting and processing—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting RC.213/18/02/C1.

FISHERIES OFFICER (male, qualified biologist with an honours degree in a natural science, preferably zoology, and experience in research work) in Tanganyika, to investigate the ecology of inland waters in relation to fish production—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting BCD.63/8/012/C1.

HEAD (man or woman, honours graduate, preferably in physics) OF THE SCIENCE DEPARTMENT—The Headmistress, Sherborne School for Girls, Dorset.

RESEARCH ASSISTANT (British Empire Cancer Campaign) (biochemist, preferably holding a Ph.D., with experience in enzymology), to work on the effects of steroids on tissue metabolism—The Department of Chemical Pathology, Royal Free Hospital, London, W.C.1.

RESEARCH OFFICERS (FISHERIES) (male, under 40, with an honours degree in natural sciences or equivalent qualification, together with at least one year's postgraduate experience in appropriate research work) IN THE CO-OPERATIVE DEVELOPMENT AND FISHERIES DEPARTMENT, Hong Kong, for duty with a research team investigating the fisheries of the South China Sea, and to supervise the work of Assistant Research Officers—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting RC.289/68/01/C1.

RESEARCH OFFICERS (with a first- or second-class honours degree in chemistry, biochemistry or chemical technology, or equivalent qualification, plus at least two years postgraduate experience) with the Federal Government of Nigeria, to carry out laboratory research and development work on Nigerian raw materials—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. E.14/1.

SENIOR LECTURER (man or woman) IN EDUCATION—The Principal, Radbrook College, Shrewsbury.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Other Countries

Department of Scientific and Industrial Research. Road Research 1960: The Report of the Road Research Board with the Report of the Director of Road Research. Pp. iv + 138 + 8 plates. (London: H.M. Stationery Office, 1961.) 7*s*. net. [258

Frank Mason and Co., Ltd. Director's Report and Statement of Accounts for the year ended 31st March 1961. Pp. 8 (London: Frank Mason and Co., Ltd., 1961.) [258

Soil Mechanics Limited. Geotechnical Pamphlet No. 7: Chemical Consolidation in Civil Engineering Practice. Pp. xii. (London: Soil Mechanics, Ltd., 1961.) [258

Privy Council. Medical Research Council Special Report Series, No. 300: Cerebral Infarction: The Role of Stenosis of the Extracranial Cerebral Arteries. By Peter O. Yates and Edward C. Hutchinson. Pp. viii + 95 + 20 plates. (London: H.M. Stationery Office, 1961.) 14*s*. net. [258

Department of Scientific and Industrial Research: Geological Survey and Museum. Geology and Ceramics: a Brief Review of the Nature, Geological Occurrence, Processing, and Principal Industrial Applications of the Rocks and Minerals used in British Ceramic Manufacture. By Dr. P. J. Adams. Pp. ii + 28. (London: H.M. Stationery Office, 1961.) 3*s*. 6*d*. net. [258

The Edinburgh School of Agriculture; The Edinburgh and East of Scotland College of Agriculture. Calendar for 1961-62. Pp. 113. (Edinburgh: The Edinburgh School of Agriculture; The Edinburgh and East of Scotland College of Agriculture, 1961) 1*s*. [258

The North of Scotland College of Agriculture. Calendar, 1961-62. Pp. 146. (Aberdeen: The North of Scotland College of Agriculture, 1961.) [258

Other Countries

Indian Forest Bulletins (New Series). Entomology. No. 171 (8). A List of Insect Pests of Forest Plants in India and the Adjacent Countries (arranged alphabetically according to the Plant Genera and Species, for the use of Forest Officers.) Part 9: List of Insect Pests of Plant Genera "S" (*Sabia* to *Syzygium*). By R. N. Mathur and Balwant Singh. Pp. 88. Rs. 7.00nP., 11*s*. No. 232: Insect Borers of Felled Timbers and Their Control. Part 3: The Dehra Dun Investigation of 1949–53. By Dr. M. L. Roonwal, Dr. P. N. Shatterjee and R. S. Thapa. Pp. 11. Rs. 1.35nP.; 3*s*. Indian Forest Records (New Series). Botany. Vol. 5, No. 2: Living Indian Gymnosperms. Part 1—(Cycadales, Ginkgoales and Coniferales). By M. B. Raizada and K.C. Sahni. Pp. iv + 73–150 + 13 plates. Rs. 12.00nP., 19*s*. Timber Mechanics. Vol. 1, No. 11: Physical and Mechanical Properties of Woods Tested at F.R.I.—Report X. By G. S. Begli and D. N. Bhatia. Pp. 171–183. Rs. 3.95nP., 6*s*. 6*d*. (Delhi: Manager of Publications, 1960 and 1961.) [258

Anné Geophysique Internationale. Participation Suisse a l'Expédition Suède/Finlande/Suisse—SFS, 1957–1959. Mesures des Parasites Atmosphériques, d'Electricité Atmosphérique et de Radioactivité de l'Air à Murchison Bay (Spitzberg), Payerne et Zurich. Par Jean Lugeon, André Junod, Pierre Wasserfallen et Jean Rieker. Pp. i + 267. (Zurich: Institut Suisse de Météorologie, 1960.) [258

Food and Agriculture Organization of the United Nations. Animal Health Yearbook, 1960. Pp. viii + 311. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1961.) 15*s*.; 3 dollars. [258

Publications de l'Institut National pour l'Etude Agronomique du Congo. Série Technique No. 63: Observations sur le Poids des Truies de la Race Yorkshire Large White. Par M. Vandenbranden. Pp. 34. (Bruxelles: Institut National pour l'Etude Agronomique du Congo, 1961.) 25 francs. [258

Indian Forest Records (New Series). Entomology. Vol. 10, No. 3: Pests of Teak and Their Control. By R. N. Mathur. Pp. 43 + 66 + plates 1–5. (Delhi: Manager of Publications, 1961.) Rs. 2; 5*s*. [258

Forschungsberichte des Landes Nordrhein-Westfalen. Nr. 1001: Die Informationsübertragung bei der Mikroskopie mit Röntgenstrahlen. Von Günther Langner. Pp. 126. (Köln und Opladen: Westdeutscher Verlag, 1961.) 37 D.M. [258

Bernice P. Bishop Museum. Bulletin No. 223: Subsistence Agriculture in Polynesia and Micronesia. By Jacques Barrau. Pp. 94. (Honolulu, Hawaii: Bernice P. Bishop Museum, 1961.) 2.25 dollars. [258

Quarterly Journal of Crude Drug Research, Vol. 1, No. 1 (1961). Pp. 40. Annual Subscription 24.75 guilders; 49*s*. 6*d*.; 6.90 dollars; 27.50 D.M.; 35 N.F. (Amsterdam: Dr. E. F. Steinmetz, 347 Keizersgracht, 1961.) [258

Institut Royal Météorologique de Belgique. Publications Série B, No. 32; Le Temps en 1960. Par R. Sneyers. Pp. 50. (Bruxelles: Institut Royal Météorologique de Belgique, 1961.) [258

United States Department of Agriculture. The European Chafer: How to Fight It. Pp. 6. (Washington, D.C.: Government Printing Office, 1961.) [258

Hypoxybiose und Mucopolysaccharid-Bildung un der Differenzierung und Pathologie der Gewebe sowie über den Zusammenhang zwischen Schilddrüsenfunktion und Mucopolysacchariden. Von Stefan Krompecher. (*Nova Acta Leopoldina*, Nr. 146, Bd. 22). (Leipzig: Johann Ambrosius Barth, 1960.) [258

Madjelis Ilmu Pengetahuan Indonesia. (Council for Sciences of Indonesia). Bulletin Nr. 3: Indeks Madjalah Ilmiah 1960. (Indonesian Scientific Periodical Index.) Pp. 89. (Djakarta: Madjelis Ilmu Pengetahuan Indonesia, 1961.) [258

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office